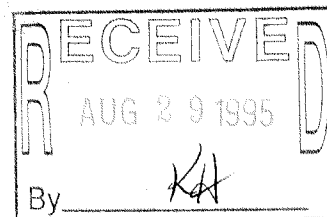


D. #32 9/13

FINAL REPORT
Year 1



DATE: August 21, 1995

SUBCONTRACT No.: XR-2-11186-1

SUBCONTRACT TITLE: Optimization of Dilute Acid Pretreatment

PRINCIPAL INVESTIGATOR: Penner/Hashimoto

SUMMARY:

The objective of subcontract XR-2-11186-1 is to determine dilute acid pretreatment conditions which provide an optimum yield of glucose and xylose from a poplar and a switchgrass feedstock. The results indicate that xylose yields of from 80 to 90% are obtainable for both feedstocks under the conditions employed in this study. Glucose yields in the range of 90 to 100% were also obtained under standard enzyme saccharification conditions. Glucose yields were correlated with the extent of xylan saccharification of the pretreated feedstock. Ethanol yields in SSF experiments averaged 75% of theoretical for poplar and 90% of theoretical for switchgrass. In general, the higher yields of xylose, glucose, and ethanol were associated with high temperature, short time, pretreatment conditions for both feedstocks.

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A handwritten signature in cursive script, appearing to read "Michael H. Penner".

8/21/95

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TASK 1.

(A) The chemical composition of the biomass starting materials is presented in table 1. The two sources having similar total glycan percentages, but differing with respect to the relative amounts of hemicellulose and cellulose. The hybrid poplar was significantly higher in lignin than the switchgrass sample, 26.9% of dry weight and 19.5% of dry weight respectively. For comparison, the chemical composition of a "quality control" sample provided by NREL is given in Table 2. The glycan fraction of the quality control sample was assayed twice based on an NREL request. Assay #1 of table 2 was done on the first quality control sample provided, assay #2 was done on the second quality control sample provided. It is our understanding, based on conversations with NREL, that samples 1 and 2 were identical.

(B) In one experiment a single pretreatment condition was used on three separate occasions with the switchgrass feedstock. The chemical composition of the retentate and prehydrolysate resulting from each run was determined (Table 3). The two most important parameters to monitor, with respect to this study, are the percent of original glucose recovered in the prehydrolyzed solid fraction and the percent of original xylose recovered in the prehydrolysate liquid. The difference in the minimum and maximum values observed for percent of original glucose retained in the prehydrolyzed solid fraction was approximately 2.1%. The maximum and minimum values observed for the percentage of original xylose recovered in the prehydrolysate liquid differed by approximately 5.8%.

(C) The enzyme preparation used in this study was tested approximately 4 weeks prior to beginning the saccharification and SSF experiments. The activity was measured in duplicate, measured activities being 83.0 and 83.5 FPU per ml (mean = 83.25 FPU/ml). Hence, the enzyme load used in saccharification and SSF experiments was based on the *T. reesei* enzyme preparation having an activity of 83.25 FPU per ml.

Table 1. Compositional Analysis of Raw Biomass Samples (Percent dry weight)

Component	Switchgrass			Poplar		
	Mean	SEM	C.V.	Mean	SEM	C.V.
Total glycans	56.6	-	-	58.2	-	-
glucan	32.2	0.29	1.1	39.8	0.01	0.0
xylan	20.3	0.26	1.6	14.8	0.03	0.2
galactan	-	-	-	-	-	-
arabinan	3.7	0.04	1.3	1.2	0.02	1.6
mannan	0.4	0.02	5.0	2.4	0.04	1.9
Klason lignin	19.5	0.3	4.0	26.9	0.26	2.4
Acid soluble lignin	3.7	0.03	2.1	2.2	0.06	6.2
Ash	7.1	0.06	2.7	1.3	0.02	5.6
Uronic acid	1.1	.01	1.0	2.4	.13	5.4
Other	12.0	-	-	9.0	-	-

Table 2. Chemical Composition of Hybrid Poplar Quality Control Sample Provided by NREL (Values Expressed as Percentage on Dry-Weight¹ Basis)

Component	Assay #1			Assay #2		
	Mean	C.V.	n	Mean	C.V.	n
glucan	42.0	1.0	3	42.7	0.3	3
xylan	13.9	1.6	3	14.0	0.6	3
galactan	0	-	3	0	-	3
arabinan	1.0	14.0	3	0.9	2.6	3
mannan	2.1	3.1	3	2.2	1.0	3
Total Glycan	59%			59.8%		
Klason lignan	25.3	0.8	3			
Acid soluble lignin	2.2	3.3	3			
Ash	1.3	3.9	3			

¹Moisture content of the sample prior to analysis was 4.2%.

Table 3. A Comparison of Three Identical Pretreatment Runs¹

Component	Feedstock ² Composition (%, dry weight basis)	Composition of Prehydrolyzed Solids (%, dry weight basis)			% of Original Component Recoverd in Prehydrolyzed Solid			% of Original Component Recovered in Prehydrolysate Liquid		
		run 1	run 2	run 3	run 1	run 2	run 3	run 1	run 2	run 3
Dry matter	100%				56.3	55.6	55.9			
glucan	31.8	50.4	49.9	50.8	89.1	87.2	89.3	15.9	16.9	18.1
xylan	20.3	3.2	3.5	3.6	8.9	9.5	10.0	82.5	83.8	88.3
galactan	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
arabinan	3.5	0.4	0.4	0.6	6.4	6.4	10.4	105.8	106.7	109.8
mannan	0.4	0.0	0.0	0.0	0.0	0.0	0.0	35.1	35.6	37.1
Ash	7.1	5.6	5.4	5.3	44.3	42.7	41.9			
Klason lignin	19.5	31.9	31.2	31.6	91.9	89.9	91.1			
Acid soluble lignin	3.7	1.6	1.6	1.5	24.3	24.3	22.8			

¹ Pretreatment condition = 0.9% acid, 160°C, 3 minutes² Feedstock = switchgrass

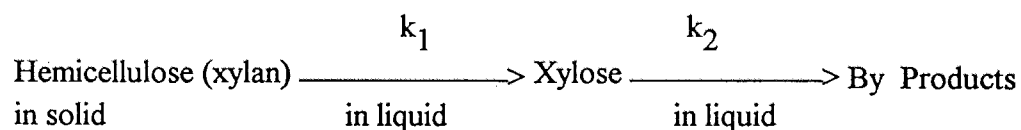
TASK 2.

(A) The matrix of pretreatment conditions used for model development is presented in Table 4. The xylose recovery data associated with each of the pretreatment conditions is given in Table 5 (switchgrass) and Table 6 (poplar). Analytical methods used for the analysis of raw and pretreated samples were based on protocols provided by NREL.

(B) Using the data described in (A) above a kinetic model was developed which was capable of predicting the amount of xylose that could be recovered in the prehydrolysate liquid following dilute acid pretreatments. Tables 7 (switchgrass) and 8 (poplar) report the maximum xylose yields which may be expected for a given acid concentration and temperature using the reactor design employed in this study. The data are presented in terms of a single time point, that corresponding to the maximum xylose yield for the given reactor conditions. The following text describes the development of the model and defines pertinent parameters. Appendix 1 contains information on the operation of the reactor. In appendix 2 we have included a representative set of figures which illustrate the fit between simulated and experimental data. Appendix 3 compares the application of a biphasic model and a single-phase model for predicting the amount of xylose remaining in pretreated solids.

Modeling the Hydrolysis Reaction of Switchgrass and Poplar

The hemicellulose hydrolysis reaction is assumed to be in general form of:



Both reactions are assumed to be first order reactions, so:

$$r = k_1 \cdot X_S - k_2 \cdot X_L \quad (I)$$

r : rate of xylose formation in liquid phase

X_S : the amount of xylan in solids residue

X_L : the amount of xylose in liquid

Having measured the amount of xylose remaining in the solids residue of each experiment at various reaction times, the following model was shown to fit the data:

$$X_S = S \cdot e^{-k_1 \cdot t} + I$$

X_S : Percentage of xylose equivalents remaining in solids residue, relative to initial weight of xylose in substrate

S : Percentage of hydrolyzable xylose

I : Percentage of unhydrolyzable xylose in substrate; $I = 100 - S$

k_1 : Reaction rate constant (min^{-1})

t : time (min)

This model conforms to our observation that under certain pretreatment conditions (especially milder conditions) some of the hemicellulose is resistant to hydrolysis (I).

Both S and k_1 are dependent upon acid concentration and reaction temperature, so the correlation between them needs to be found. The following model represents this relationship, and constants were derived by using a *linear regression analysis* when acid concentration, temperature, and corresponding S values were known:

$$S = \alpha \cdot T^{\beta} \cdot C^{\gamma} \quad (II)$$

T: Reaction temperature (°K)
C: Acid concentration (wt%)

The reaction rate constant was assumed to have an *Arrhenius* type relationship with respect to temperature and acid concentration as follows:

$$k_1 = A_{01} \cdot C^{n_1} \cdot e^{-E_1/R.T} \quad (III)$$

A_{01} : Pre-exponential factor
 E_1 : Activation energy (KJ/mol)
R: 8.3143×10^{-3} (KJ/mol.°K)

Having different C, T, and k values, and using a *linear regression analysis* on the linearized form of the above equation, A_{01} , n_1 and E_1 were calculated.

Therefore, the following set of equations can be used to estimate the percentage of xylose remaining in a substrate after acid hydrolysis:

$$XS = S \cdot e^{-k_1 \cdot t} + I \quad (IV)$$

$$I = 100 - S$$

$$k_1 = A_{01} \cdot C^{n_1} \cdot e^{-E_1/R.T}$$

$$S = \alpha \cdot T^{\beta} \cdot C^{\gamma}$$

Since some of the xylose produced may be converted to other byproducts (such as furfural) during hydrolysis, the amount of xylose loss should also be modeled.

Xylose Loss Model:

Following model is derived to determine the amount of xylose loss:

$$L = (100 - X_S) \cdot (1 - e^{-k_2 \cdot t}) \quad (V)$$

L: Xylose loss, percentage of xylose which is converted to byproducts, such as furfural

X_S: Percentage of xylose equivalents remaining in solids residue

k₂: Rate constant of (Xylose \longrightarrow By products) reaction

t: time (min)

and:

$$K_2 = A_0 \cdot 2 \cdot C^{n_2} \cdot e^{-E_2/R \cdot T} \quad (VI)$$

$$R = 8.3143 \times 10^{-3} \text{ KJ/mol} \cdot ^\circ\text{K}$$

T: temperature ($^\circ$ K)

C: acid concentration (wt%)

For switchgrass: $A_0 \cdot 2 = 6.7 \times 10^{14}$, $n_2 = 1.9$, $E_2 = 138.46 \text{ KJ/mol}$

For poplar: $A_0 \cdot 2 = 9.9 \times 10^{13}$, $n_2 = 0.313$, $E_2 = 129.23 \text{ KJ/mol}$

All constants are derived by using *linear regression analysis* on known and measured terms. It is of interest that the two feedstocks have different constants associated with xylose degradation. This was not anticipated at the beginning of the study

since we assumed xylose degradation would be feedstock independent. At this point we can only speculate on the reason for the different constants. One of the principal reasons for the differences may be related to the neutralizing capacity of each feedstock. All of our modeling is based on the amount of acid injected into the reaction mixture, not on the actual hydrogen ion activity in the reaction mixtures. This means that if the feedstocks differ in their neutralizing capacity, and we expect they do, then reaction mixtures assumed to have equivalent hydrogen ion activities will actually not be equivalent.

To obtain the percentage of net amount of xylose in the liquid stream (relative to initial xylose in substrate) based on equation (I); equations (IV) and (V) were consolidated, and the following model was derived:

$$X_L = (100 - X_S) \cdot e^{-k_2 t} \quad (VII)$$

X_L : xylose in liquid (% relative to total amount of xylose in the starting material)

X_S : xylose remaining in solids residue (%)

The attached table shows all required equation constants for both poplar and switchgrass. In order to determine the maximum amount of X_L , at a given temperature and acid concentration, the first derivative of the above equation (VII) is taken and set to zero:

$$dX_L / dt = 0$$

hence,

$$t_{\max} = (-1/k_1) \cdot \ln(k_2 / k_1 + k_2)$$

	<u>Switchgrass</u>	<u>Poplar</u>
A_{01}	4.57×10^{10}	1×10^{18}
E_1 (KJ/mol)	86.91	148.75
n_1	0.968	0.707
<hr/>		
α	0.235	1.44
β	0.983	0.69
γ	0.11	0.049
<hr/>		
A_{02}	6.7×10^{14}	9.9×10^{13}
E_2 (KJ/mol)	138.46	129.23
n_2	1.9	0.313

Figures showing the percentage of xylose remaining in the retained solids (X_s), as well as percent of xylose lost (L) vs. time are included in appendix 2. Each of these figures correspond to a particular combination of temperature and acid concentration. Lines show the values derived from the global (unifying) model, equation (IV), and symbols represent the experimental data. Replicates and reproducibility data are shown with asterisks.

Since most workers used a biphasic equation to model the hydrolysis reaction of biomass, we also tried that for our data. This model can be represented as:

$$X = X_f \cdot e^{-k_f t} + X_s \cdot e^{-k_s t}$$

in which both k_f and k_s are assumed to have an Arrhenius type relationship with respect to temperature:

$$k = A \cdot e^{-E/RT}$$

the pre-exponential factor, A , is dependent upon acid concentration (C):

$$A = A_0 \cdot C^n$$

The results for poplar, show that the trends of k_f and k_s with respect to temperature and acid concentration were quite reasonable. However, percentage of fast (or slow) reacting portion of xylose, X_f (or X_s), did not show a rational, or at least justifiable, behavior with temperature or acid concentration. For switchgrass, the trend of X_f with respect to temperature and acid concentration is not as bad as of poplar, and is predictable to some extent. k_f increased with temperature increase, but decreased with an increase in acid concentration, which is unexpected. k_s did not show any justifiable behavior with respect to acid concentration or temperature.

In the results shown by Grohman et al. (1985) for Aspen wood, X_f is reasonably constant with respect to temperature, and in case of wheat straw this value changes drastically (from 17% to 53%). However, even in the latter case, they did not attempt to find a relationship between X_f and temperature.

Maloney and Chapman (1985), found X_f (Z, as they call it) "virtually independent of reaction conditions", and took the average value and derived a unifying model.

In our case, X_f ranged from 69% to 97% for poplar, and from 51% to 94% for switchgrass. However, an average value of X_f was assumed, and the global model was derived. The correlation coefficients (R^2) were slightly lower than our proposed single phase model (see below). It's worth noticing that in the proposed single phase model:

$$X = S \cdot e^{-k \cdot t} + I$$

the trend of S with respect to reaction conditions is quite consistent with chemical kinetic theory.

In summary, for the single phase model, both S and k values are dependent upon reaction conditions (T and C). This dependency can be predicted by using equations (II), (III), and (VI). In contrast, we found no way to relate X_f to T and/or C in the biphasic model for poplar. For switchgrass, the trend of k_s does not seem to comply with an Arrhenius type relationship. Graphs showing the experimental data and the fit achieved by both global models are shown in appendix 2. The correlation coefficients for both global models were relatively close:

	R^2	
	<u>Single phase model</u>	<u>Biphasic model</u>
Poplar	0.982	0.974
Switchgrass	0.986	0.981

Following table shows derived constants for the biphasic model for both poplar and switchgrass, the constants are to be used with the following set of equations:

$$X = X_f \cdot e^{-k_f t} + X_s \cdot e^{-k_s t}$$

$$k_f \text{ or } k_s = A_o \cdot C^n \cdot e^{-E/RT}$$

Biphasic Model

	<i>Switchgrass</i>	<i>Polplar</i>
X_f (average)	76.76%	83.80%
A_o	2×10^{21}	3.3×10^{21}
k_f : E (KJ/mol)	169.97	176.73
n	2.1	0.46
A_o	2.3×10^{23}	2.5×10^{22}
k_s : E (KJ/mol)	201.69	192
n	2.77	1.83

Table 5 - Composition of Switchgrass PHS and PH								
Pretreatment Conditions	% of Original Dry Matter in PHS	Wt% Glucan of PHS	% of Original Glucan in PHS	% of Original Glucan in PH	Wt.% of Xylan of PHS	% of Original Xylan in PHS	% of Original Xylan in PH	Total % of Xylan Recovered
0 min., 140 C, 1.2%	74.5	38.4	89.0	nd	21.8	80.0	nd	
0.5 min., 180 C, 0.6%	56.1	50.6	88.2	16.6	5.0	13.8	78.5	92.3
0.5 min., 180 C, 0.9%	54.7	51.5	87.5	17.8	5.0	13.4	84.7	98.1
0.5 min., 180 C, 1.2%	52.6	49.7	81.3	17.9	2.2	5.7	80.8	86.5
1 min., 160 C, 0.6%	62.9	43.9	85.9	nd	11.8	36.6	nd	
1 min., 160 C, 0.9%	59.7	46.1	85.6	nd	8.6	25.3	nd	
1 min., 160 C 1.2%	59.1	46.1	84.8	nd	8.1	23.5	nd	
1 min., 160 C, 1.2%	55.8	50.3	87.3	16.4	4.4	12.1	81.3	93.4
1 min., 180 C, 0.6%	53.0	50.4	84.0	16.2	4.3	11.4	80.2	91.6
1 min., 180 C, 0.9%	52.7	51.3	84.1	nd	3.0	7.7	nd	
1 min., 180 C, 0.9%	51.6	52.5	84.4	17.1	1.7	4.5	88.3	92.8
1 min., 180 C, 1.2%	51.5	51.6	82.6	nd	2.2	5.5	nd	
2 min., 160 C, 0.9%	56.8	47.0	83.1	nd	6.8	19.0	nd	
2 min., 160 C, 1.2%	55.3	48.3	83.1	nd	4.9	13.3	nd	
2 min., 180 C, 0.6%	54.3	51.2	87.5	16.4	5.2	13.3	72.5	85.8
2 min., 180 C, 0.9%	50.0	52.3	82.1	19.2	2.3	5.7	77.1	82.8
2 min., 180 C, 1.2%	49.2	51.7	79.9	21.3	1.9	4.7	72.2	76.9
3 min., 140 C, 0.6%	66.1	41.3	84.8	nd	17.7	57.7	nd	
3 min., 140 C, 0.9%	62.6	45.6	88.7	nd	12.9	39.6	nd	
3 min., 140 C, 1.2%	62.0	44.5	85.8	nd	12.0	36.6	nd	
3 min., 140 C, 1.2%	58.8	46.7	85.5	nd	10.8	31.4	nd	
3 min., 160 C, 0.6%	59.2	45.5	83.8	nd	10.0	29.1	nd	
3 min., 160 C, 0.9%	55.0	49.4	84.6	nd	5.3	14.3	nd	
3 min., 160 C, 0.9%	55.3	51.9	89.2	15.9	3.3	9.1	83.6	92.7
3 min., 160 C, 0.9%	54.5	51.5	87.2	16.9	3.6	9.6	84.9	94.6
3 min., 160 C, 0.9%	55.8	51.5	89.3	18.1	3.8	10.4	89.4	99.8
3 min., 160 C, 1.2%	59.0	47.0	86.2	nd	9.0	26.1	nd	
3 min., 180 C, 0.6%	53.3	51.8	85.9	nd	3.8	9.9	nd	
3 min., 180 C, 0.9%	50.6	52.7	82.9	nd	2.4	5.9	nd	
3 min., 180 C, 1.2%	48.4	52.4	79.6	22.1	1.5	3.7	71.2	74.8
5 min., 140 C, 0.6%	64.4	42.4	84.8	nd	15.0	47.7	nd	

Table 5 - Composition of Switchgrass PHS and PH - Continued								
Pretreatment Conditions	% of Original Dry Matter in PHS	Wt% Glucan of PHS	% of Original Glucan in PHS	% of Original Glucan in PH	Wt% Xylan of PHS	% of Original Xylan in PHS	% of Original Xylan in PH	Total % Xylan Recovered
5 min., 140 C, 0.9%	59.8	46.7	86.8	nd	10.7	31.4	nd	
5 min., 140 C, 1.2%	56.5	48.6	85.4	nd	8.3	23.2	nd	
5 min., 160 C, 0.6%	55.3	48.9	84.0	nd	5.5	14.9	nd	
5 min., 160 C, 0.9%	52.6	52.9	86.5	16.7	2.3	6.0	84.7	90.7
5 min., 160 C, 0.9%	55.4	48.6	83.8	nd	5.5	15.0	nd	
5 min., 160 C, 1.2%	51.7	51.4	82.6	nd	2.8	7.2	nd	
5 min., 180 C, 0.6%	49.5	51.4	79.1	nd	2.4	5.8	nd	
5 min., 180 C, 0.9%	49.1	49.0	74.8	nd	1.6	3.9	nd	
5 min., 180 C, 1.2%	49.1	45.0	68.7	nd	4.4	10.7	nd	
15 min., 140 C, 0.6%	58.8	46.6	85.2	nd	10.3	29.7	nd	
15 min., 140 C, 0.9%	56.2	49.9	87.3	15.8	5.1	14.1	78.2	92.3
15 min., 140 C, 0.9%	58.6	45.7	83.4	nd	9.5	27.3	nd	
15 min., 140 C, 1.2%	53.3	46.3	76.7	nd	10.8	28.3	nd	
30 min., 140 C, 0.6%	57.1	48.4	86.7	16.5	8.4	24.0	72.1	96.0
30 min., 140 C, 0.9%	61.3	44.3	85.3	14.7	10.8	32.9	58.9	91.8
30 min., 140 C, 1.2%	54.4	49.6	83.9	nd	4.6	12.3	nd	
60 min., 140 C, 0.6%	55.7	49.1	85.9	16.4	6.9	19.0	71.5	90.5
60 min., 140 C, 0.9%	54.4	49.9	84.4	nd	3.9	10.4	nd	
60 min., 140 C, 1.2%	53.3	50.3	83.4	nd	3.4	9.0	nd	

Table 6 - Composition of Poplar PHS and PH								
Pretreatment Conditions	% of Original Dry Matter in PHS	Wt.% Glucan of PHS	% of Original Glucan in PHS	% of Original Glucan in PH	Wt. % Xylan of PHS	% of Original Xylan in PHS	% of Original Xylan in PH	Total % Xylan Recovered
0.5 min., 170 C, 1.2%	72.1	52.6	95.3	4.4	2.5	12.1	75.3	87.4
0.5 min., 180 C, 0.9%	69.4	54.3	94.8	5.1	1.9	8.8	80.2	89.1
0.5 min., 180 C, 1.2%	66.9	54.9	92.2	6.4	1.3	5.8	78.9	84.8
0.53 min., 180 c, 1.1%	67.6	53.8	91.4	5.6	1.6	7.5	80.3	87.8
0.56 min., 180 C, 1.0%	73.0	54.9	100.7	5.8	1.5	7.2	82.7	89.9
0.56 min., 180 C, 1.0%	74.9	54.8	103.2	5.7	1.5	7.4	81.1	88.5
0.6 min., 180 C, 0.9%	75.3	54.5	103.1	5.7	1.5	7.7	81.8	89.5
0.6 min., 180 C, 0.9%	75.4	53.3	101.1	5.0	1.8	9.4	77.1	86.5
1 min., 160 C, 0.6%	82.2	48.4	100.0	2.1	9.5	52.6	45.1	97.8
1 min., 160 C, 0.6%	78.3	51.8	101.9	1.8	4.4	23.4	50.9	74.2
1 min., 160 C, 0.9%	75.6	53.0	100.8	1.8	3.5	18.0	56.7	74.7
1 min., 160 C, 0.9%	76.0	55.4	105.8	3.1	5.0	25.5	64.1	89.7
1 min., 160 C, 1.2%	73.2	55.1	101.4	1.8	2.3	11.4	44.2	55.6
1 min., 160 C, 1.2%	75.7	54.1	102.9	3.1	4.9	25.2	62.6	87.8
1 min., 180 C, 0.6%	68.8	56.1	97.0	6.6	0.9	4.2	90.6	94.8
1 min., 180 C, 0.9%	64.6	55.4	90.0	6.6	1.2	5.1	83.0	88.2
1 min., 180 C, 0.9%	66.2	58.3	97.0	7.2	0.3	1.3	78.8	80.1
1 min., 180 C, 1.2%	64.4	56.4	91.3	8.5	0.2	0.9	76.0	76.9
1.2 min., 170 C, 1.2%	67.6	54.0	91.7	5.7	1.4	6.5	82.7	89.2
1.3 min., 170 C, 1.1%	69.7	53.1	93.1	5.4	1.3	5.9	80.4	86.3
2 min., 160 C, 0.6%	74.6	53.8	100.9	0.0	2.8	13.8	64.5	78.3
2 min., 160 C, 0.9%	72.1	54.3	98.5	3.6	2.1	10.1	70.4	80.5+192
2 min., 160 C, 1.2%	70.5	54.9	97.3	4.2	1.4	6.7	72.7	79.4
2 min., 180 C, 0.6%	67.3	57.1	96.5	4.5	0.6	2.8	72.9	75.7
2 min., 180 C, 0.9%	65.9	56.3	93.2	7.5	0.4	1.8	62.6	64.5
2 min., 180 C, 1.2%	61.9	57.2	89.1	5.3	0.0	0.0	72.2	72.2
2 min., 170 C, 1.2%	67.2	54.4	91.9	6.2	1.3	5.8	81.1	86.9
3 min., 160 C, 0.6%	74.1	53.1	98.8	3.4	2.4	11.8	69.0	80.8
3 min., 160 C, 0.9%	69.5	54.3	94.8	4.7	1.3	6.1	76.1	82.1
3 min., 160 C, 0.9%	71.1	53.5	95.7	3.9	3.2	15.5	71.3	86.8
3 min., 160 C, 1.2%	68.1	54.0	92.5	1.8	0.8	3.6	76.5	80.1
3 min., 180 C, 0.6%	64.9	57.4	93.6	5.9	0.4	1.8	68.1	69.9

Table 6 - Composition of Poplar PHS and PH - Continued								
Pretreatment Conditions	% of Original Dry Matter in PHS	Wt.% Glucan of PHS	% of Original Glucan in PHS	% of Original Glucan in PH	Wt. % Xylan of PHS	% of Original Xylan in PHS	% of Original Xylan in PH	Total % Xylan Recovered
3 min., 180 C, 0.9%	63.0	56.7	89.9	9.8	0.0	0.0	69.5	69.5
3 min., 180 C, 1.2%	61.2	54.5	83.8	11.7	0.0	0.0	57.7	57.7
5 min., 140 C, 0.6%	87.8	44.1	97.2	1.2	11.2	66.0	24.7	90.7
5 min., 160 C, 0.6%	73.2	52.8	97.1	3.4	3.5	17.3	66.9	84.2
5 min., 160 C, 0.9%	69.1	54.8	95.1	4.6	2.0	9.1	73.3	82.4
5 min., 160 C, 0.9%	69.5	53.8	94.0	4.8	1.9	8.9	77.9	86.8
5 min., 160 C, 1.2%	68.1	55.3	94.8	5.2	2.0	9.1	70.2	79.3
5 min., 180 C, 0.6%	63.5	56.5	90.2	7.7	0.5	2.2	55.1	57.3
5 min., 180 C, 0.9%	59.1	54.0	80.2	13.4	0.0	0.0	42.3	42.3
5 min., 180 C, 1.2%	57.7	51.5	74.7	19.4	0.0	0.0	43.5	43.5
15 min., 140 C, 0.6%	79.1	49.0	97.6	1.9	7.8	41.6	48.9	90.5
15 min., 140 C, 0.9%	74.0	51.2	95.3	3.5	5.5	27.3	64.2	91.5
15 min., 140 C, 0.9%	74.4	51.7	96.8	3.0	4.3	21.4	65.0	86.4
15 min., 140 C, 1.2%	71.2	52.6	94.1	3.0	4.4	21.0	66.8	87.8
30 min., 140 C, 0.6%	73.5	53.7	99.2	1.7	3.0	14.7	34.4	49.1
30 min., 140 C, 0.9%	70.6	56.6	100.4	3.1	2.2	10.4	55.7	66.1
30 min., 140 C, 1.2%	69.5	55.2	96.4	4.3	1.6	7.6	69.9	77.5
60 min., 140 C, 0.6%	71.4	54.3	97.4	2.8	2.8	13.5	64.8	78.3
60 min., 140 C, 0.9%	67.7	54.8	93.3	5.0	1.3	6.0	68.0	73.9
60 min., 140 C, 1.2%	67.1	56.0	94.4	5.2	1.3	5.9	66.8	72.7

Table 7. Predicted Maximum Xylose Yields from Switchgrass for Dilute Acid Pretreatment Under Specified Acid/Temperature Conditions.

Xylose yield (% of theoretical)	Acid (wt%)	Temp (°C)	Time* (minutes)
81.1	0.6	140	20.8
82.4	0.6	150	10.7
83.8	0.6	160	5.7
84.7	0.6	170	3.1
85.4	0.6	180	1.7
82.3	0.7	140	17.4
83.6	0.7	150	9.0
84.7	0.7	160	4.8
85.6	0.7	170	2.6
86.2	0.7	180	1.4
83.3	0.8	140	15.0
84.5	0.8	150	7.7
85.6	0.8	160	4.1
86.4	0.8	170	2.2
86.9	0.8	180	1.2
84.2	0.9	140	13.1
85.4	0.9	150	6.8
86.4	0.9	160	3.6
87.1	0.9	170	1.9
87.5	0.9	180	1.1
84.9	1.0	140	11.6
86.1	1.0	150	6.0
87.0	1.0	160	3.2
87.6	1.0	170	1.7
87.9	1.0	180	0.9
85.6	1.1	140	10.4
86.8	1.1	150	5.4
87.6	1.1	160	2.8
88.1	1.1	170	1.5
88.3	1.1	180	0.8
86.3	1.2	140	9.5
87.3	1.2	150	4.8
88.1	1.2	160	2.6
88.5	1.2	170	1.4
88.6	1.2	180	0.8

* Represents the optimum reaction time for the specified acid concentration and temperature as predicted by the kinetic model described in text.

Table 8. Predicted Maximum Xylose Yields from Poplar for Dilute Acid Pretreatment Under Specified Acid/Temperature Conditions.

Xylose yield (% of theoretical)	Acid (wt%)	Temp (°C)	Time* (minutes)
76.9	0.6	140	31.1
79.3	0.6	150	11.5
81.7	0.6	160	4.5
84.0	0.6	170	1.8
86.1	0.6	180	0.8
78.0	0.7	140	28.3
80.5	0.7	150	10.5
82.2	0.7	160	4.1
85.0	0.7	170	1.6
87.2	0.7	180	0.7
79.0	0.8	140	26.2
81.4	0.8	150	9.7
83.7	0.8	160	3.8
86.0	0.8	170	1.5
81.8	0.8	180	0.6
79.8	0.9	140	24.4
82.3	0.9	150	9.0
84.6	0.9	160	3.5
86.8	0.9	170	1.4
88.9	0.9	180	0.6
80.6	1.0	140	22.9
83.0	1.0	150	8.5
85.3	1.0	160	3.3
87.5	1.0	170	1.3
89.7	1.0	180	0.6
81.3	1.1	140	21.6
83.7	1.1	150	8.0
86.0	1.1	160	3.1
88.2	1.1	170	3.3
90.3	1.1	180	0.5
81.9	1.2	140	20.5
84.3	1.2	150	7.6
86.6	1.2	160	2.9
88.8	1.2	170	1.2
90.9	1.2	180	0.5

* Represents the optimum reaction time for the specified acid concentration and temperature as predicted by the kinetic model described in text.

TASK 3.

Eight "near optimum" pretreatment conditions were chosen for a further pretreatment experiments in which prehydrolysate xylose yield and glucose yield following enzymatic saccharification of the prehydrolyzed solid fraction were determined. The "near optimum conditions were chosen based on an initial evaluation of the matrices of data presented in Tables 5 and 6. The data associated with each of the eight near-optimum pretreatment conditions for switchgrass is contained in Table 9. The corresponding data for poplar is given in Table 10. Each of these tables includes measurements of xylose recovered in the prehydrolysate and the percent of theoretical yield of glucose at 12h and at the completion of the enzymatic saccharification (168 hr). Considering the switchgrass data, the maximum yield of sugars was obtained for the pretreatment at 1.2% acid, 180°C for 0.5 min. Maximum yields for switchgrass being ~92% of theoretical xylose and ~100% of theoretical glucose. With respect to the poplar data, the maximum yields of xylose were all near 80% of theoretical. In general, glucose yeilds from pretreated poplar were in the range of 75 to 100% of theoretical. Time courses of glucose production from the pretreated switchgrass and poplar preparations are presented in figures 1&2 and 3&4, respectively. Included in each figure is a times course for glucose production from cellulose (alpha-cellulose, Sigma product # C-8002) under equivalent saccharification conditions.

Table 9. Comparative Sugar Yields from Switchgrass Following Dilute Acid Pretreatments

Treatment Conditions			Sugar Yields		
Acid (%)	Temp (°C)	Time (min)	% of Original Xylose Recovered in Prehydrolysate Liquid	Glucose Yield @ 12 hr. (% of theoretical)	Glucose Yield @ 168 hr. (% of theoretical)
0.6	180	0.5	78.5	64	93
0.9	140	15.0	78.2	55	85
0.9	160	3.0	86.0	72	95
0.9	160	5.0	84.7	73	91
0.9	180	0.5	84.7	69	94
0.9	180	1.0	88.3	74	93
1.2	160	1.0	81.3	62	93
1.2	180	0.5	91.8	77	101

FIGURE 1.

Saccharification Yield of Switchgrass

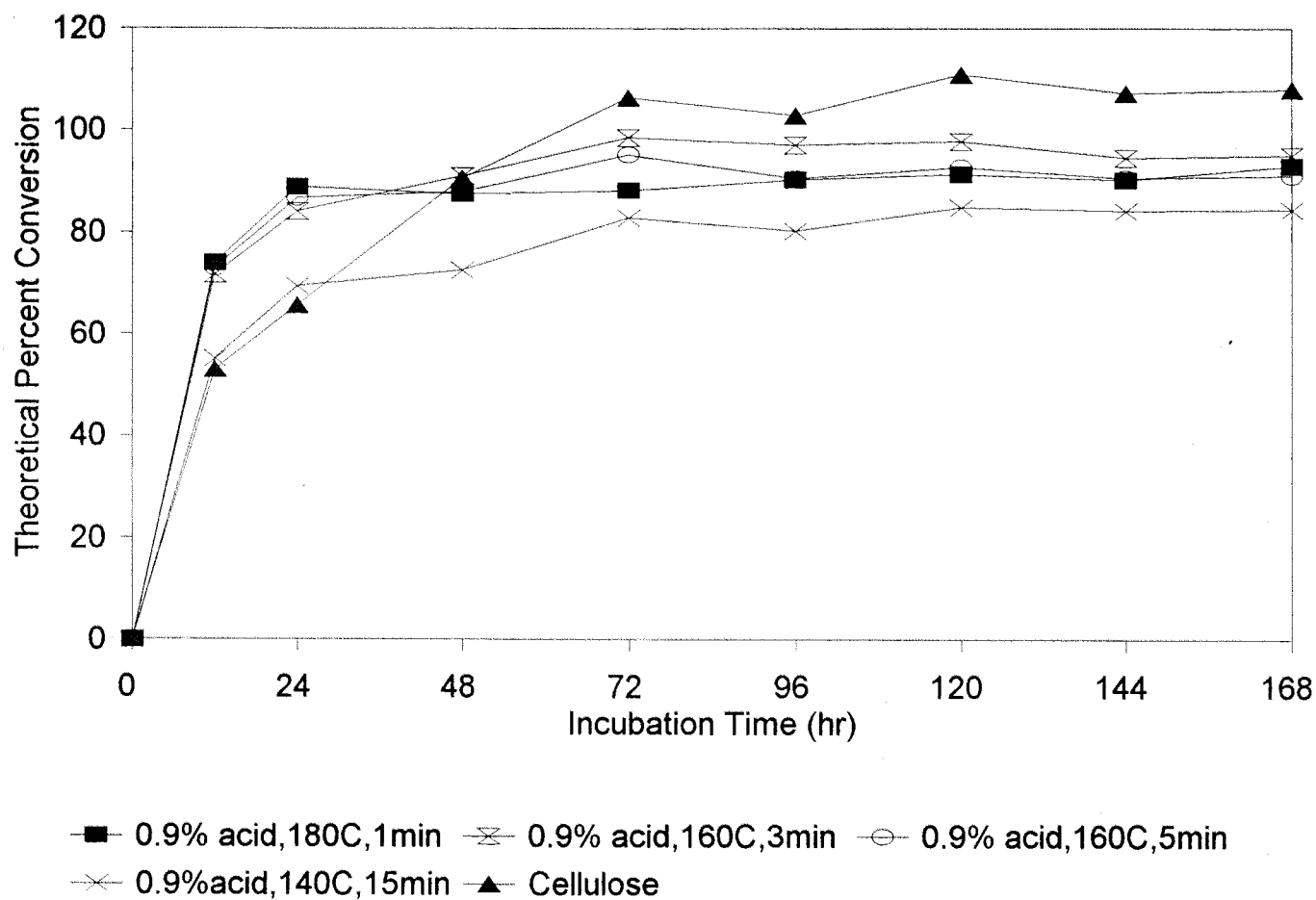


FIGURE 2.

Saccharification Yield of Switchgrass

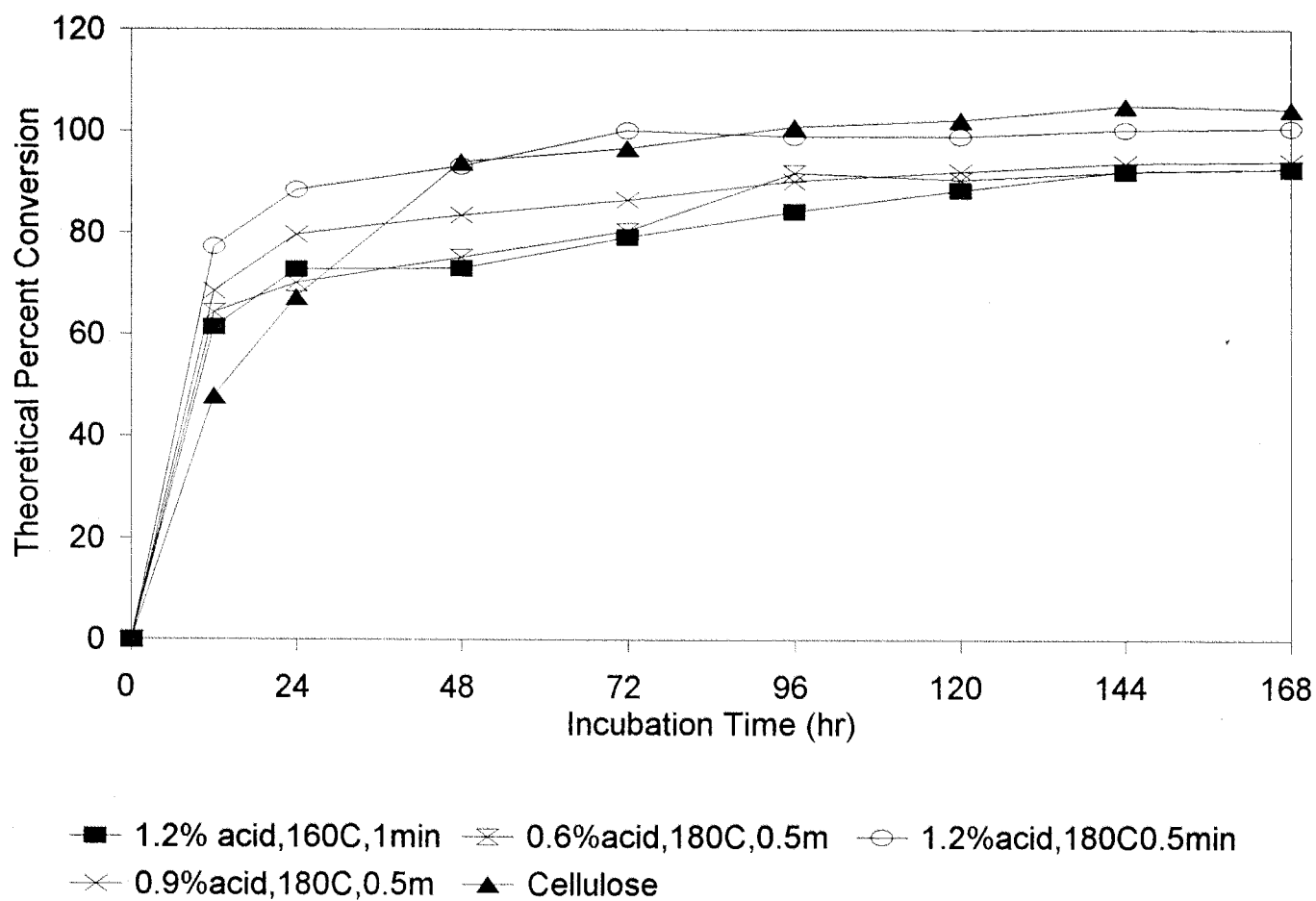


Table 10. Comparative Sugar Yields from Poplar Following Dilute Acid Pretreatments

Treatment Conditions			Sugar Yields		
Acid (%)	Temp (°C)	Time (min).	% of Original Xylose Recovered in Prehydrolysate Liquid	Glucose Yield @ 12 hr. (% of theoretical)	Glucose Yield @ 168 hr. (% of theoretical)
0.9	180	0.6	79.5	45	96
1.0	180	0.56	81.9	63	101
1.1	170	1.3	80.4	31	87
1.1	180	0.53	80.3	48	107
1.2	170	0.5	75.3	27	75
1.2	170	1.2	82.7	37	91
1.2	170	2.0	81.1	40	93
1.2	180	0.5	78.9	44	96

FIGURE 3.

Saccharification Yield of Poplar

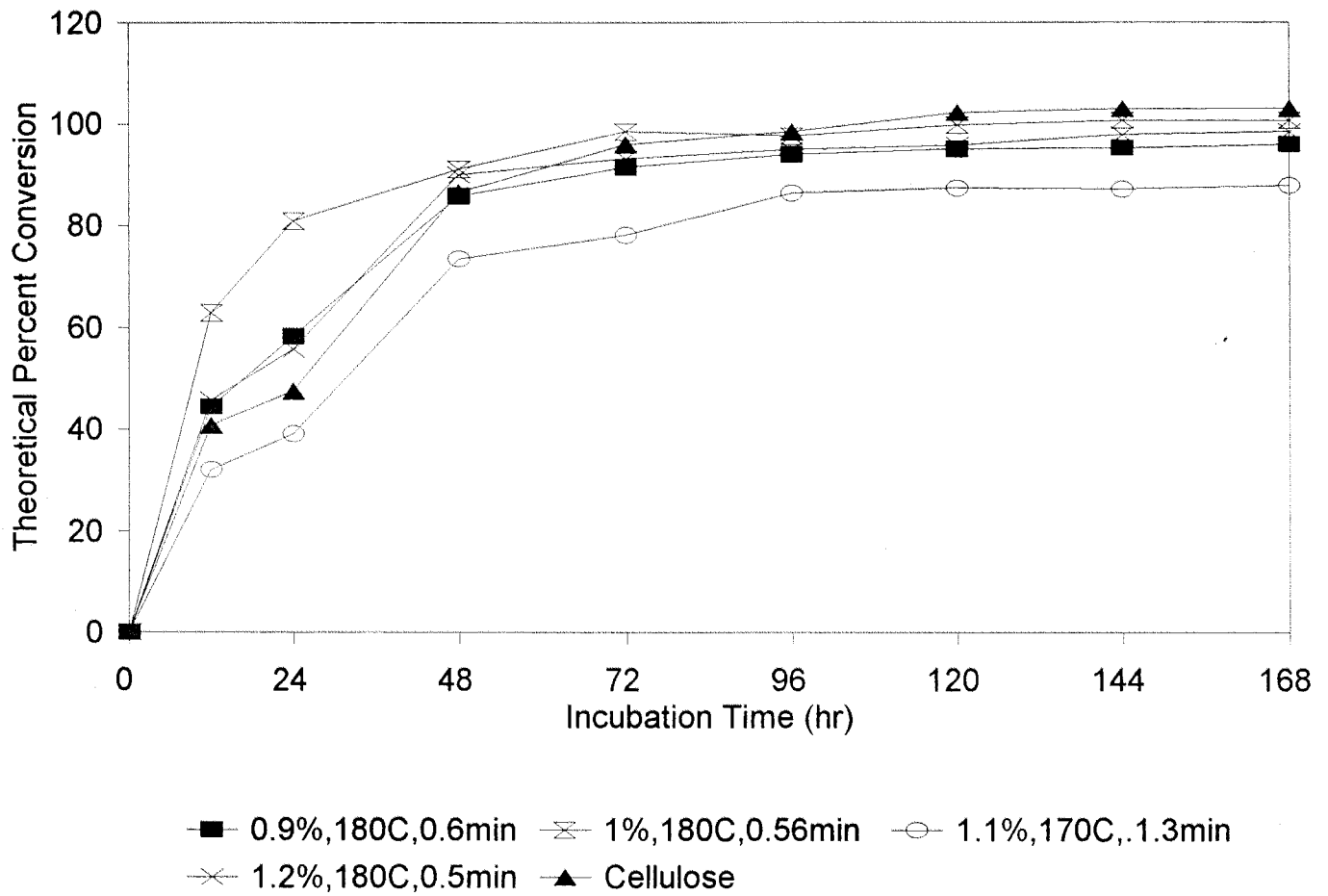
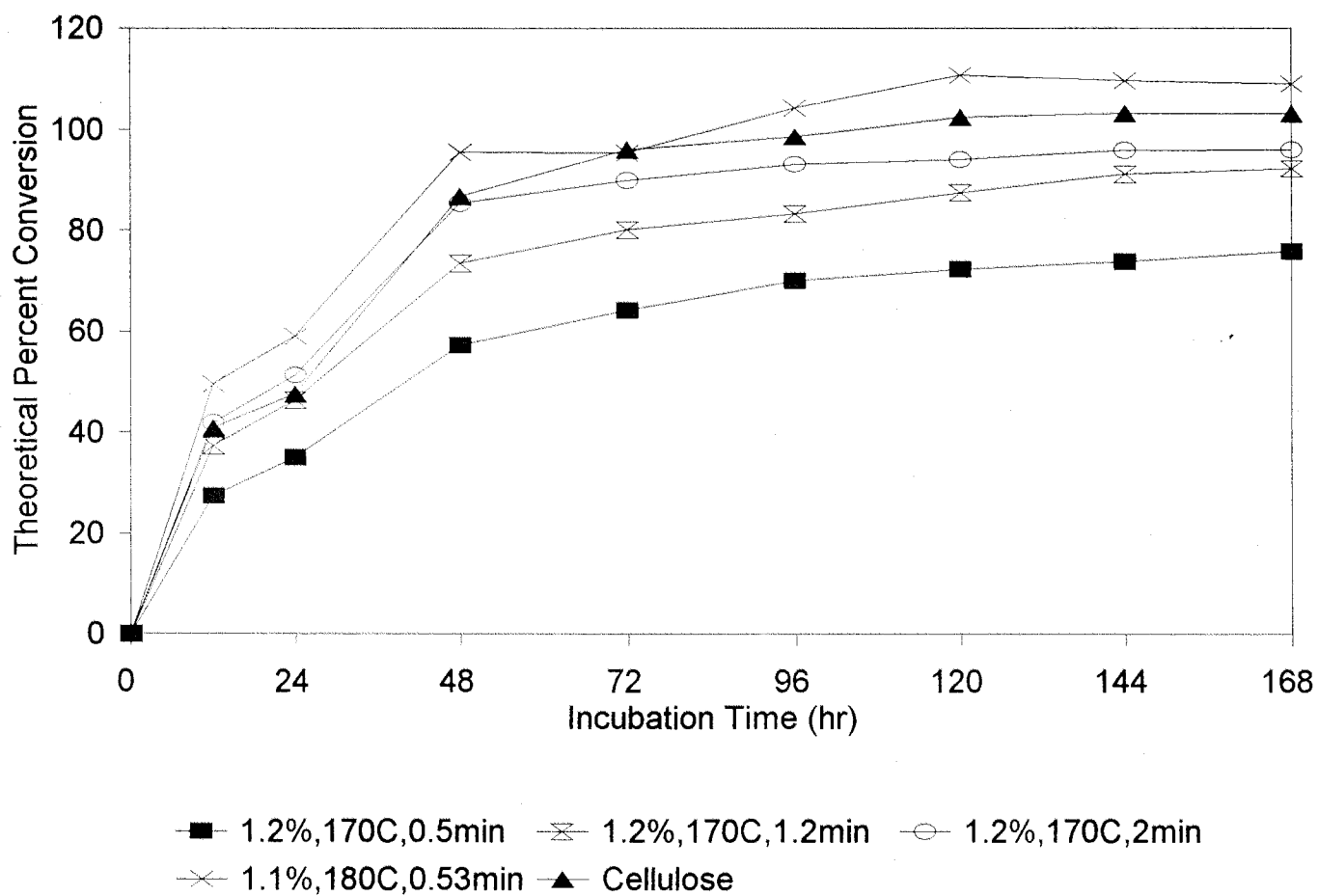


FIGURE 4.

Saccharification Yield of Poplar



TASK 4.

Two pretreatment conditions were chosen for each feedstock for further experimental work in which mass balance calculations were included for each feedstock and each pretreated residue was tested under NREL defined SSF conditions. The two pretreatments used for switchgrass SSF experiments were 0.9% acid, 180°C, 0.5 min. and 1.2% acid, 180°C, 0.5 min. The two pretreatments used for poplar SSF experiments were 0.9% acid, 180°C, 0.6 min. and 1.0% acid, 180°C, 0.56 min. The average time course of ethanol yield (% of theoretical) under SSF conditions for each of the pretreated feedstocks is presented in Fig. 5. Figures 6, 7, and 8 illustrate the time courses for ethanol, glucose, and cellobiose production from each feedstock under SSF conditions, respectively. For comparative purposes, figures 5 - 8 include time courses for product yields from cellulose (alpha-cellulose, Sigma product # C-8002). Ethanol yields from the pretreated switchgrass feedstocks averaged 90% of theoretical. Ethanol yields from the pretreated poplar feedstocks averaged 75% of theoretical. The lower ethanol yields for poplar, relative to switchgrass, were not consistent with the essentially equivalent glucose yields from the two feedstocks in enzyme saccharification experiments (see Task 3). Further experiments are required to determine the reason for the different ethanol yields. It is possible that the observed differences are related to the higher nonspecific adsorption of enzyme to poplar solids or to the production of a fermentation byproduct, specific to poplar, which is inhibitory to the cellulolytic enzymes. Tables 11 and 12 contain the mass balance data associated with the pretreatments for switchgrass and poplar, respectively.

FIGURE 5.

Ethanol Yield of Biomass

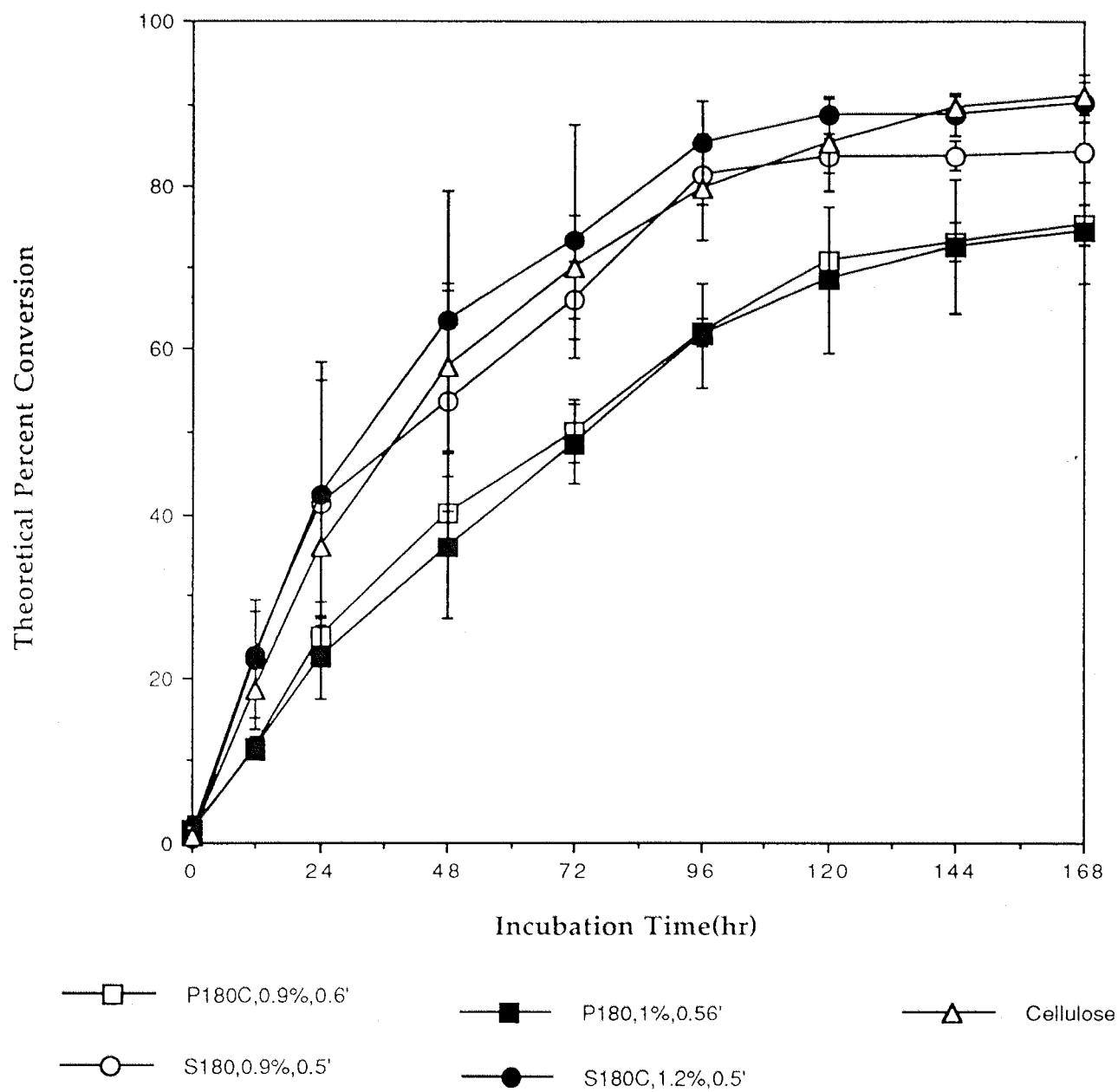


FIGURE 6.

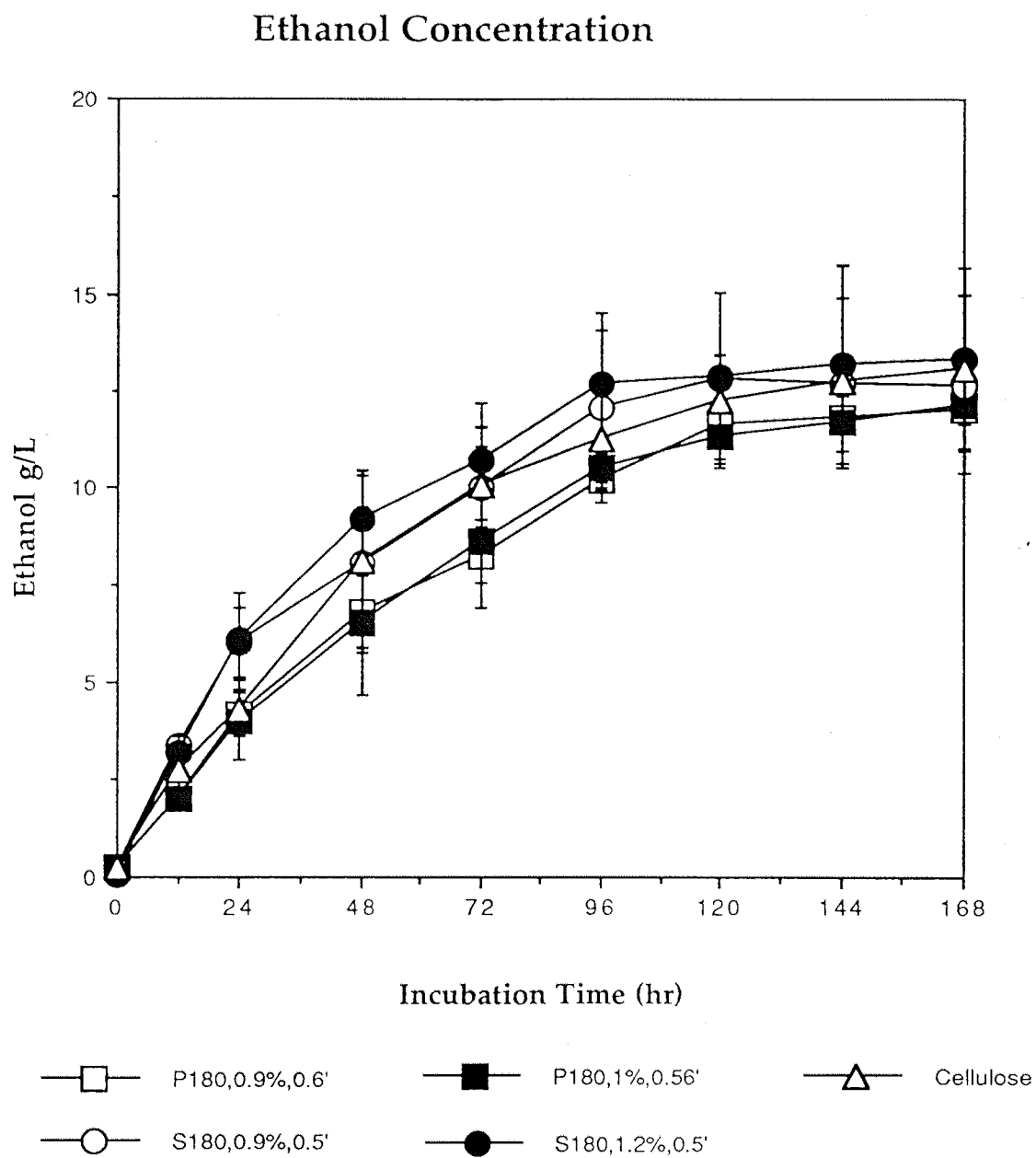


FIGURE 7.

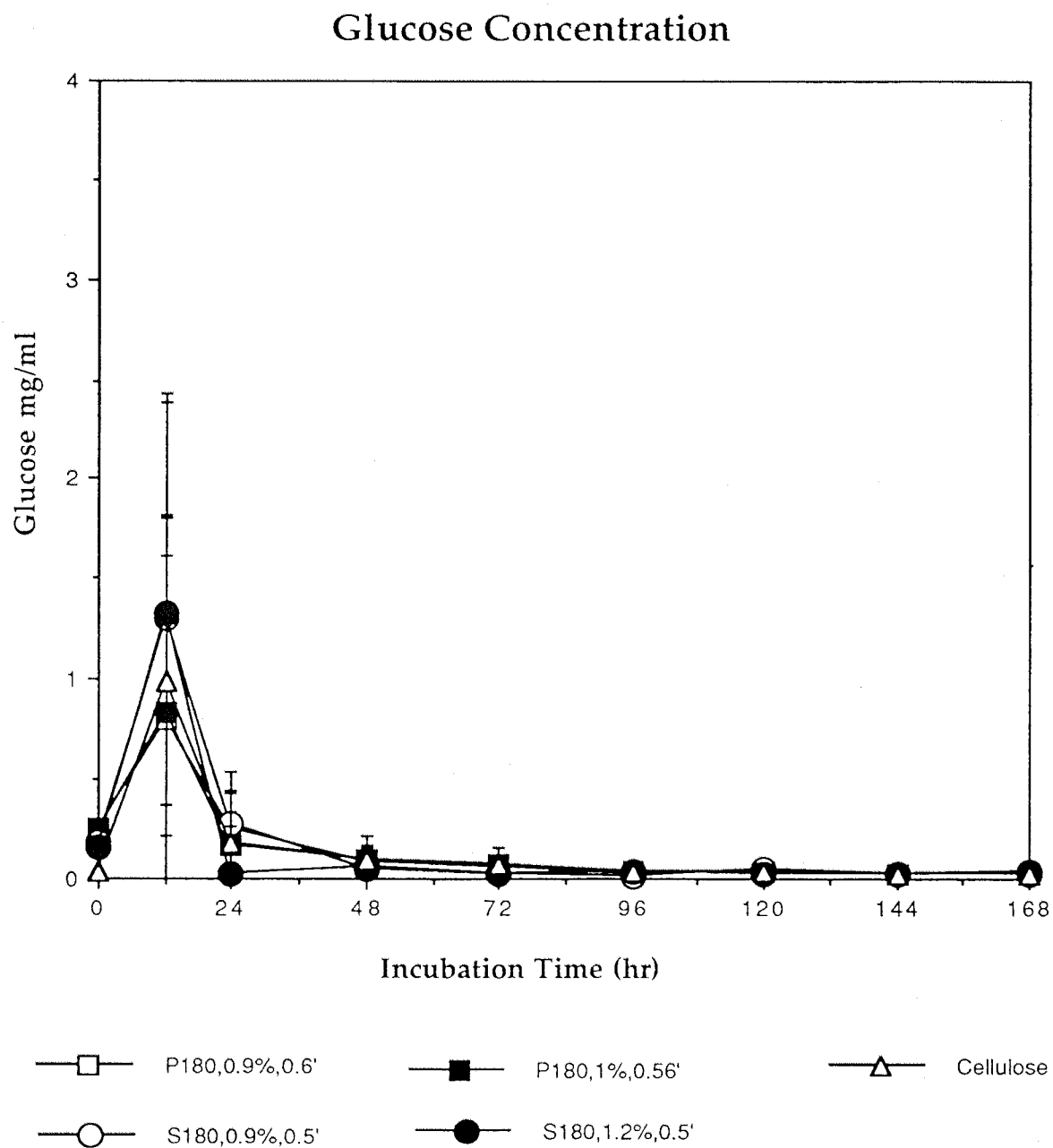


FIGURE 8.

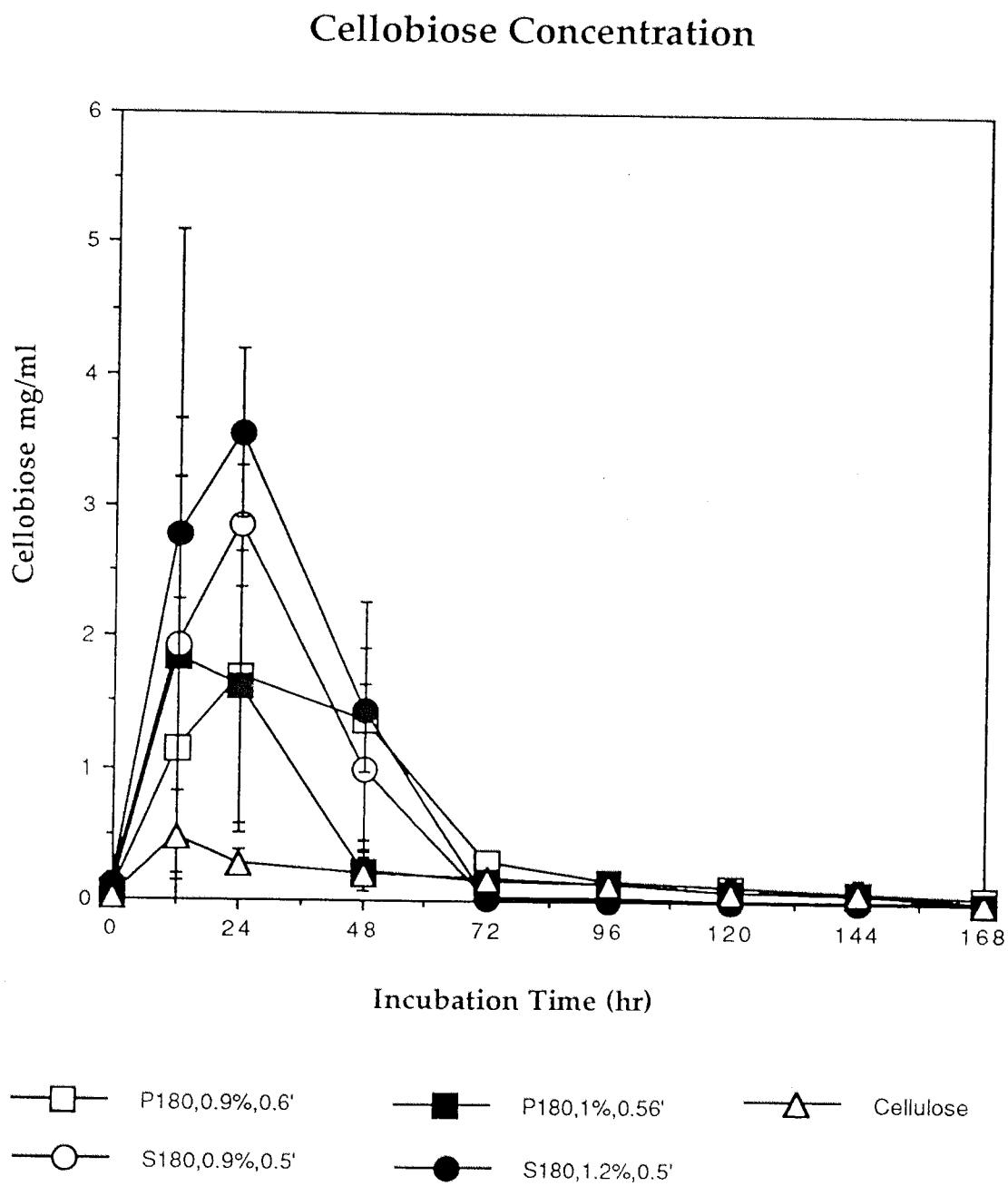


Table 11. Mass Balance Data for Two Switchgrass Pretreatments¹

Component	Feedstock ² Composition (% dry wt. basis)	Composition of Prehydrolyzed Solids (%, dry wt. basis)		% of Original Component Recovered in Prehydrolyzed Solid		% of Original Component Recovered in Prehydrolysate Liquid	
		0.9%, 180 °C, 0.5 min.	1.2%, 180 °C, 0.5 min	0.9%, 180 °C, 0.5 min.	1.2%, 180 °C, 0.5 min	0.9%, 180 °C, 0.5 min.	1.2%, 180 °C, 0.5 min
Dry matter	100			54.7	52.6	35.7	43.8
Glucan	32.2	51.5	49.7	87.4	81.3	17.8	17.9
Xylan	20.3	5.0	2.2	13.4	5.7	84.7	80.8
Galactan	0.0	-	-	-	-	-	-
Arabinan	3.7	-	-	-	-	106.0	68.4
Mannan	0.4	-	-	-	-	-	-
Ash	7.1	5.6	7.8	43.1	57.9	-	71.5
Klason Lignin	19.5	31.9	34.9	89.5	94.2	NA	NA
Acid Soluble Lignin	3.7	1.6	1.1	23.7	17.6	-	102.0
Uronic acid	1.1	0.32	0.34	15.9	17.3	58.8	61.2

¹ Pretreatment conditions = 0.9% acid, 180 °C, 0.5 minutes and 1.2% acid, 180 °C, 0.5 minutes.

² Feedstock = Switchgrass

NA = not applicable

Table 12. Mass Balance Data for Two Poplar Pretreatments¹

Component	Feedstock ² Composition (% dry wt. basis)	Composition of Prehydrolyzed Solids (%, dry wt. basis)		% of Original Component Recovered in Prehydrolyzed Solid		% of Original Component Recovered in Prehydrolysate Liquid	
		1.0%, 180 °C, 0.56 min.	0.9%, 180 °C, 0.6 min	1.0%, 180 °C, 0.56 min.	0.9%, 180 °C, 0.6 min	1.0%, 180 °C, 0.56 min.	0.9%, 180 °C, 0.6 min
Dry matter	100			74.0	75.4	30.0	30.1
Glucan	39.8	54.9	53.9	102.0	102.1	5.8	5.5
Xylan	14.8	1.5	1.7	7.3	8.6	81.9	79.5
Galactan	0	-	-	-	-	-	-
Arabinan	1.2	-	-	-	-	-	-
Mannan	2.4	0.8	0.8	24.7	25.1	51.9	52.3
Ash	1.3	0.2	0.2	11.3	11.6	185.9	188.8
Klason Lignin	26.9	35.9	35.9	98.8	100.6	NA	NA
Acid Soluble Lignin	2.2	1.4	1.5	47.1	51.4	98.0	97.1
Uronic Acid	2.4	1.2	1.1	37.0	34.6	72.0	64.7
Furfural Xylose Equivalents	-	-	-	-	-	13.0	10.9

¹Pretreatment conditions = 0.9% acid, 180 °C, 0.6 minutes and 1.0% acid, 180 °C, 0.56 minute.²Feedstock = Poplar

NA = not applicable

Appendix 1

Procedure for Operating the Reactor and Preparing Material for Analysis

All reactions were carried out in a Parr reactor, model#4563. This 600-ml reactor of 316 stainless steel is equipped with glass liner, cooling coil, and impeller type mixer. Other pretreatment specifications are as follows:

Reaction initiation: Aqueous substrate suspensions are brought to the desired reaction temperature prior to acid addition (~15-20 minutes). The acid solution used to initiate the reaction is heated to the reactor set point temperature in an external reservoir. To initiate the reaction approximately 20 ml (± 1 ml depending on the concentration of the stock acid solution) of the preheated acid is injected into the reactor at 15 psi above the vapor pressure within the reactor. Injection time is ~ 3 seconds.

Temperature control: A Parr, model# 4831 controller with heater output connected to a heating mantel and cooling output connected to a solenoid valve regulating flow through internal cooling coil (temperature regulated at $\pm 5^{\circ}\text{C}$).

Reaction termination: Shut off power to heating mantle, begin continuous flow of tap water through internal cooling coil, simultaneously spraying external surface of reactor with tap water. Cooling time for 180°C to 100°C is <90 seconds.

The main steps of each experiment are as follows:

I. Reactor preparation

- a. Weigh water and substrate in glass liner
- b. Slide liner into reactor and seal reactor
- c. Weigh sulfuric acid solution in syringe
- d. Load acid solution into acid injector
- e. Weigh syringe again.
The difference between two weights of steps c and e would be the net weight of injected acid.
- f. Place reactor into holder
- g. Connect heater, thermocouple wire, agitator, pressure supply, and cooling water apparatus
- h. Adjust regulator pressure to 15 psi above reactor pressure

II. Reaction

- a. Turn on the thermostat
- b. Turn on cooling water faucet
- c. Measure and adjust the flow rate of cooling water passing through internal cooling coil, to 20 ml/min
- d. Set the thermostat to desired temperature
- e. Turn on the mixer
- f. Turn on the chart recorder
- g. When desired temperature is reached, inject the acid while releasing some pressure from the reactor
- h. After reaction time is completed, turn on cooling water valve to cool reactor

- i. Take the mantle off
- j. Cool off outside of reactor by running cold water on the reactor

III. Preparing material for analysis

A. Filter preparation

- a. Weigh the top part of a two-piece plastic Buchner funnel
- b. Place Whatman #5 filter paper in the Buchner funnel
- c. Weigh the funnel and paper together

B. Collecting the Pretreated Substrate

- a. Release pressure and remove reactor lid
- b. Rinse substrate off reactor lid, thermocouple, and cooling loop with distilled water while slowly lifting lid out of reactor
- c. Slowly lift glass liner out of the reactor while rinsing outside of liner with distilled water
- d. Pour substrate/water suspension from reactor into tared filter-paper-containing Buchner funnel
- e. Filter slurry through Buchner funnel using aspirator
- f. Slowly rinse pretreated substrate with 1500 ml of distilled water
- g. After liquid ceases to drip from filtrate, record total amount of liquid in filter flask, and save approximately 125 ml of this liquid
- h. Dry retentate with filter paper and top part of Buchner funnel in oven at 45°C for 24 hours

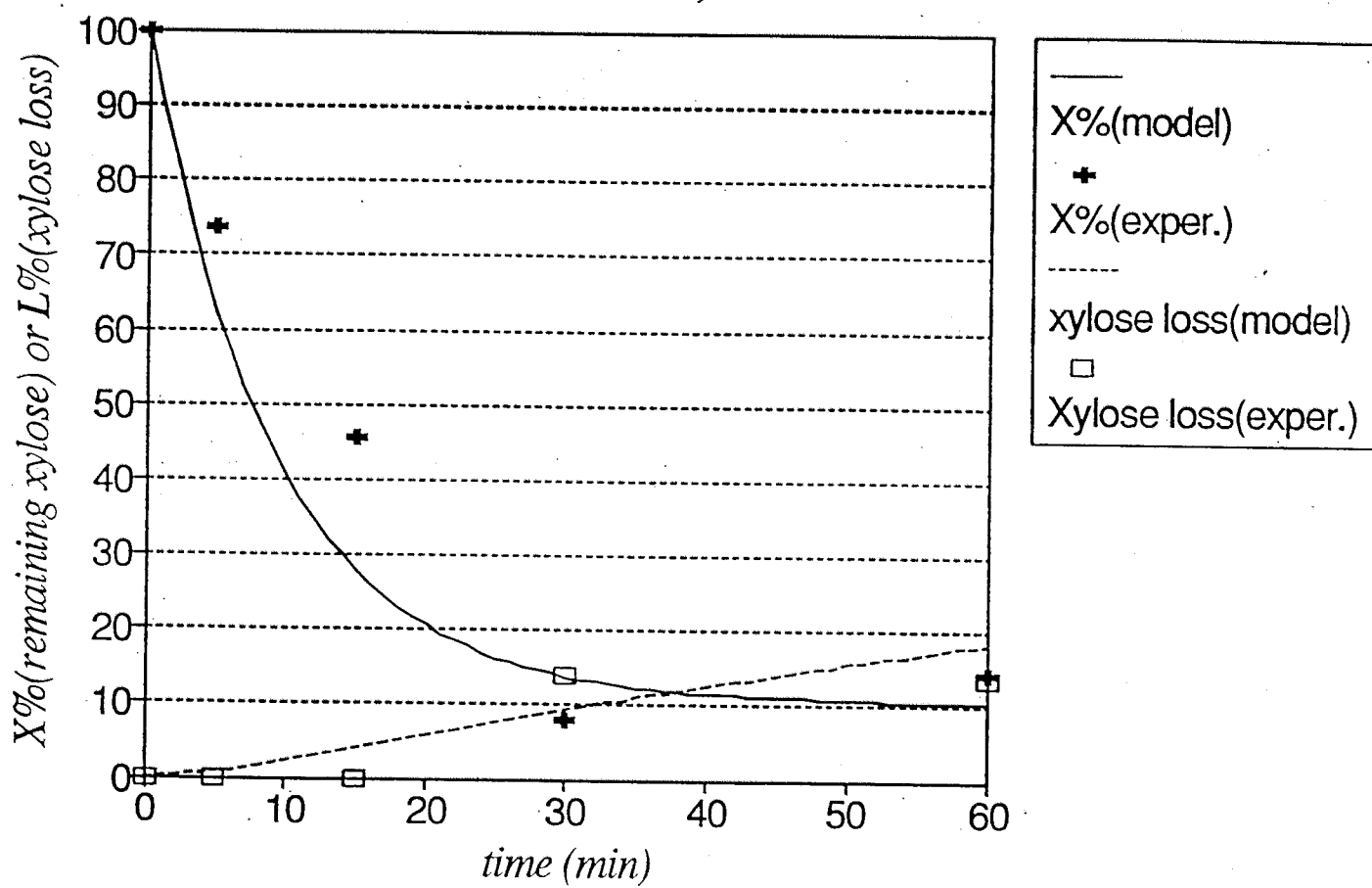
Appendix 2

The following set of figures illustrate the fit between simulated data generated by the kinetic model described under “Task 2B” and the experimental data described under “Task 2A”. The experimental data were percentages of xylose remaining in the solids residue taken from Tables 5 (Switchgrass) and 6 (Poplar).

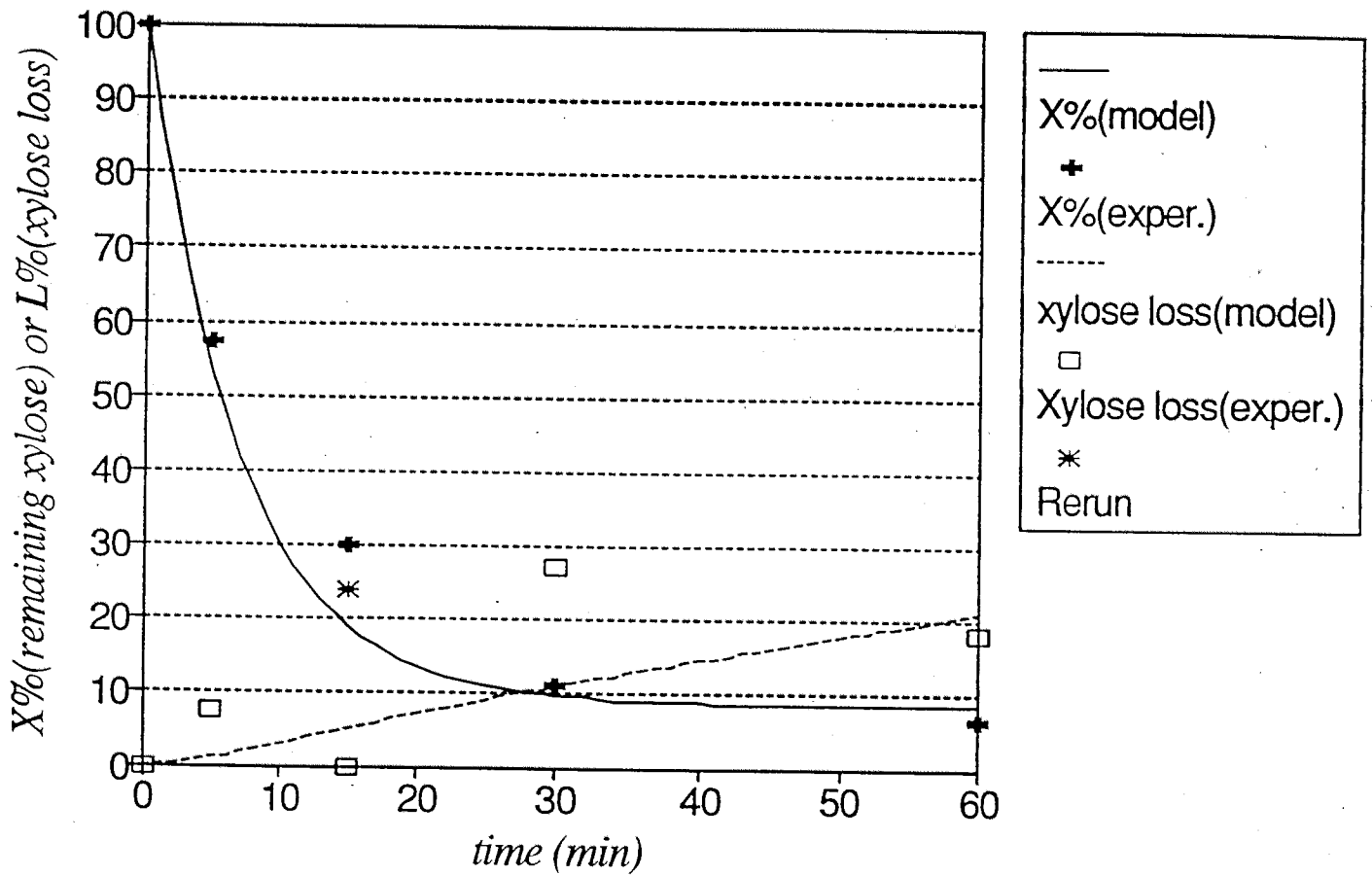
The experimental values of xylose loss (%) were calculated as:

$$100 - [\% \text{ xylose remaining in the solids residue} + \% \text{ xylose in the prehydrolysate liquid}]$$

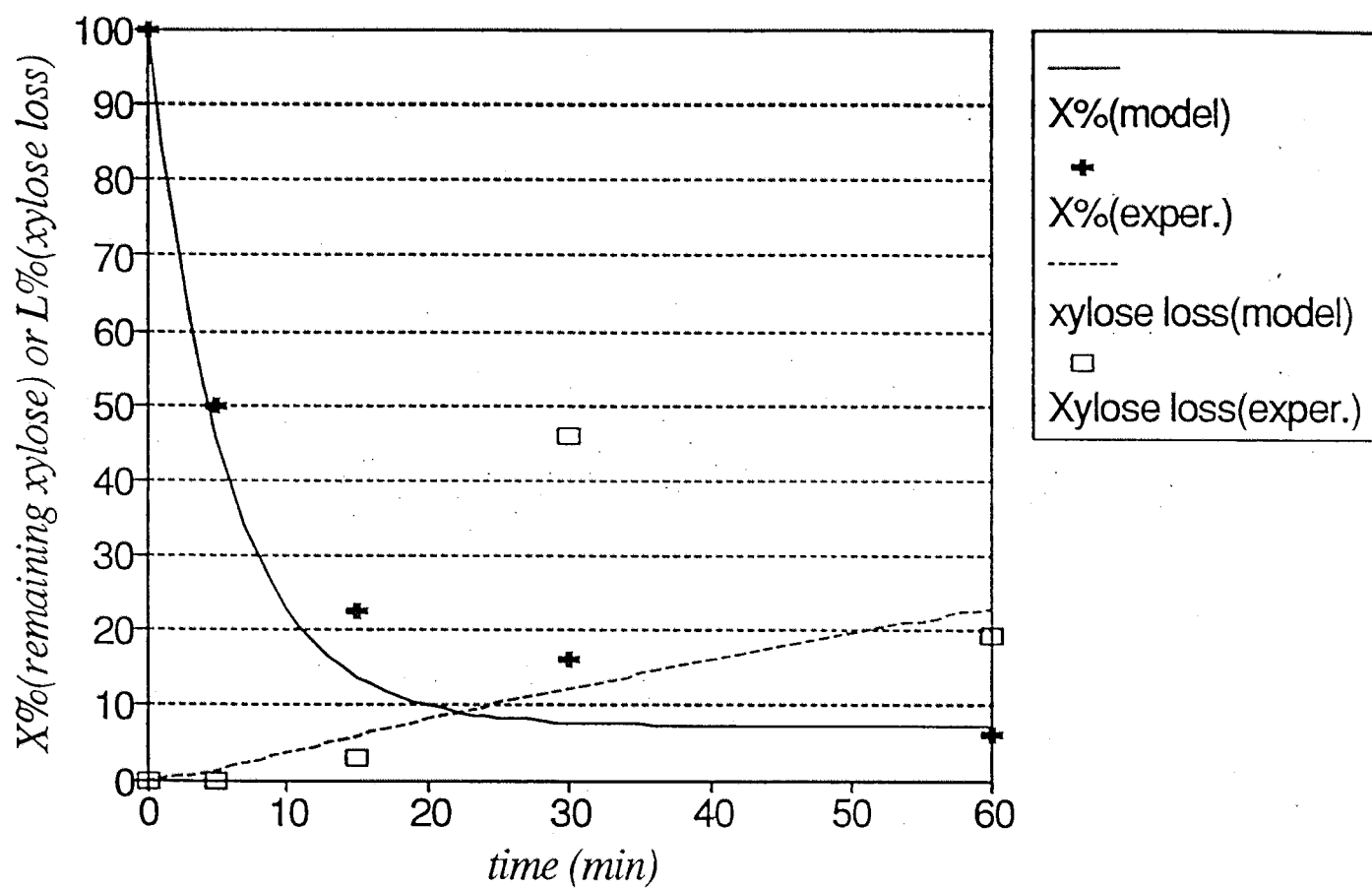
Poplar
140 C, 0.6%



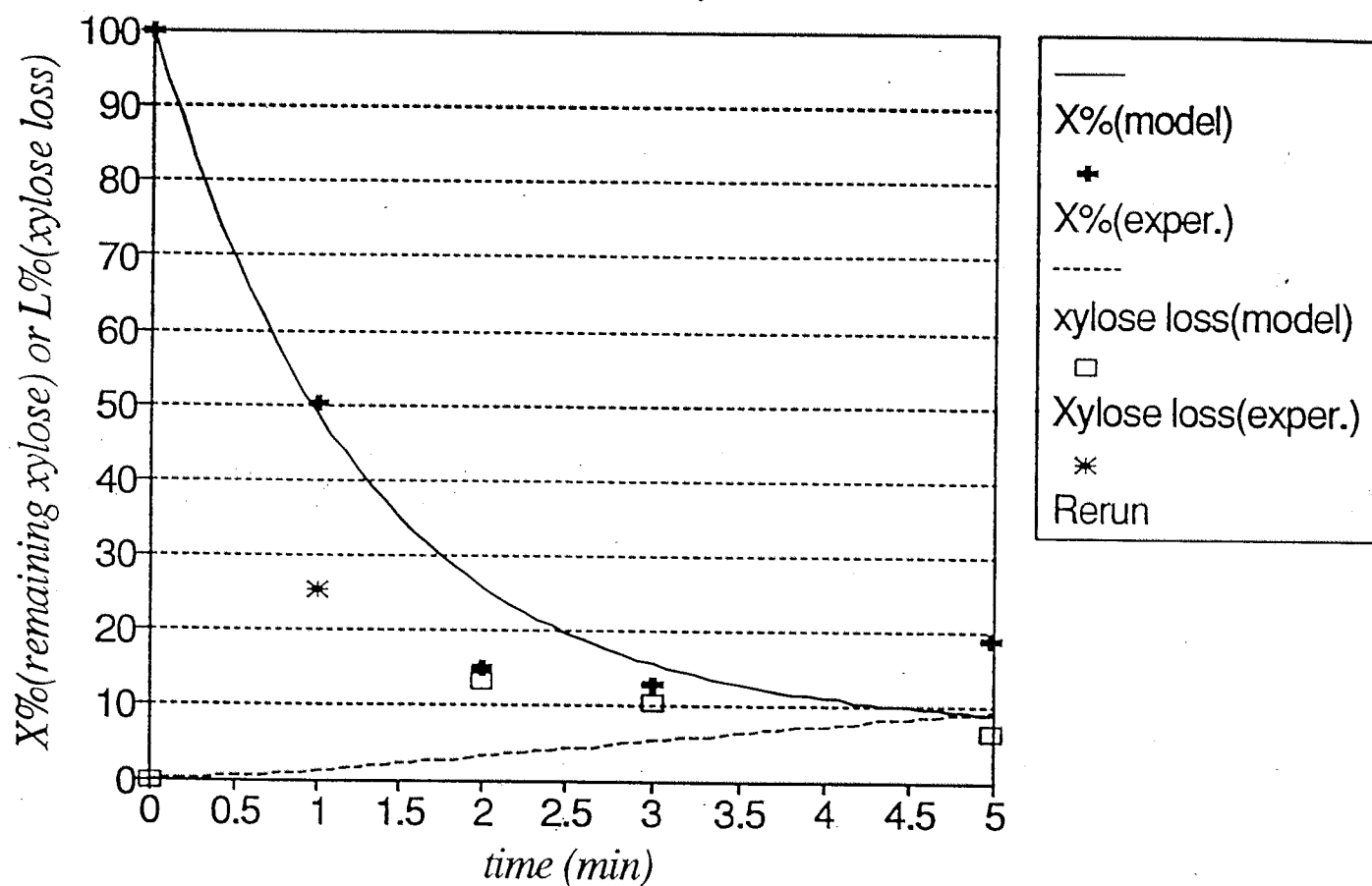
Poplar
140 C, 0.9%



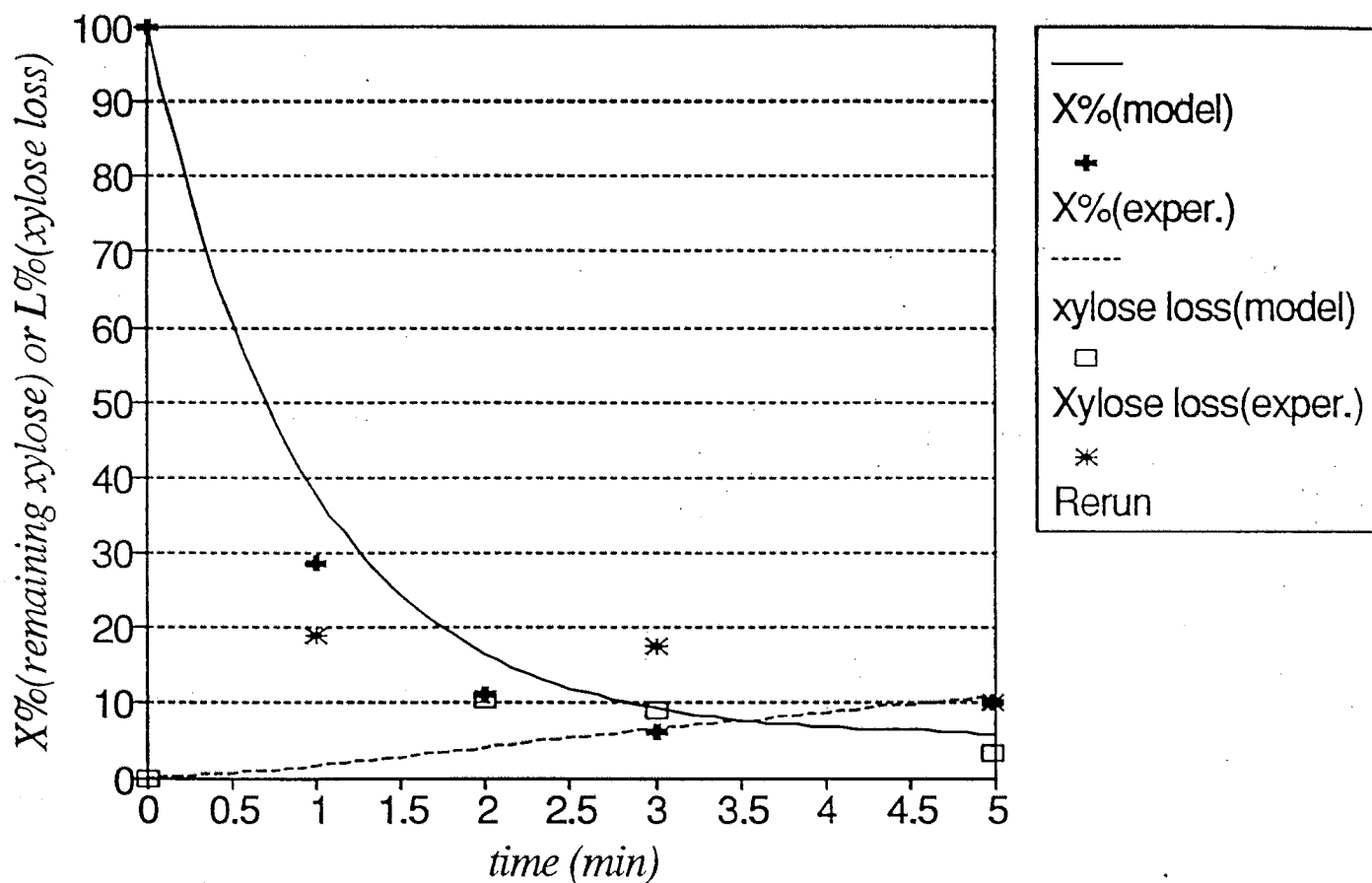
Poplar
140 C, 1.2%



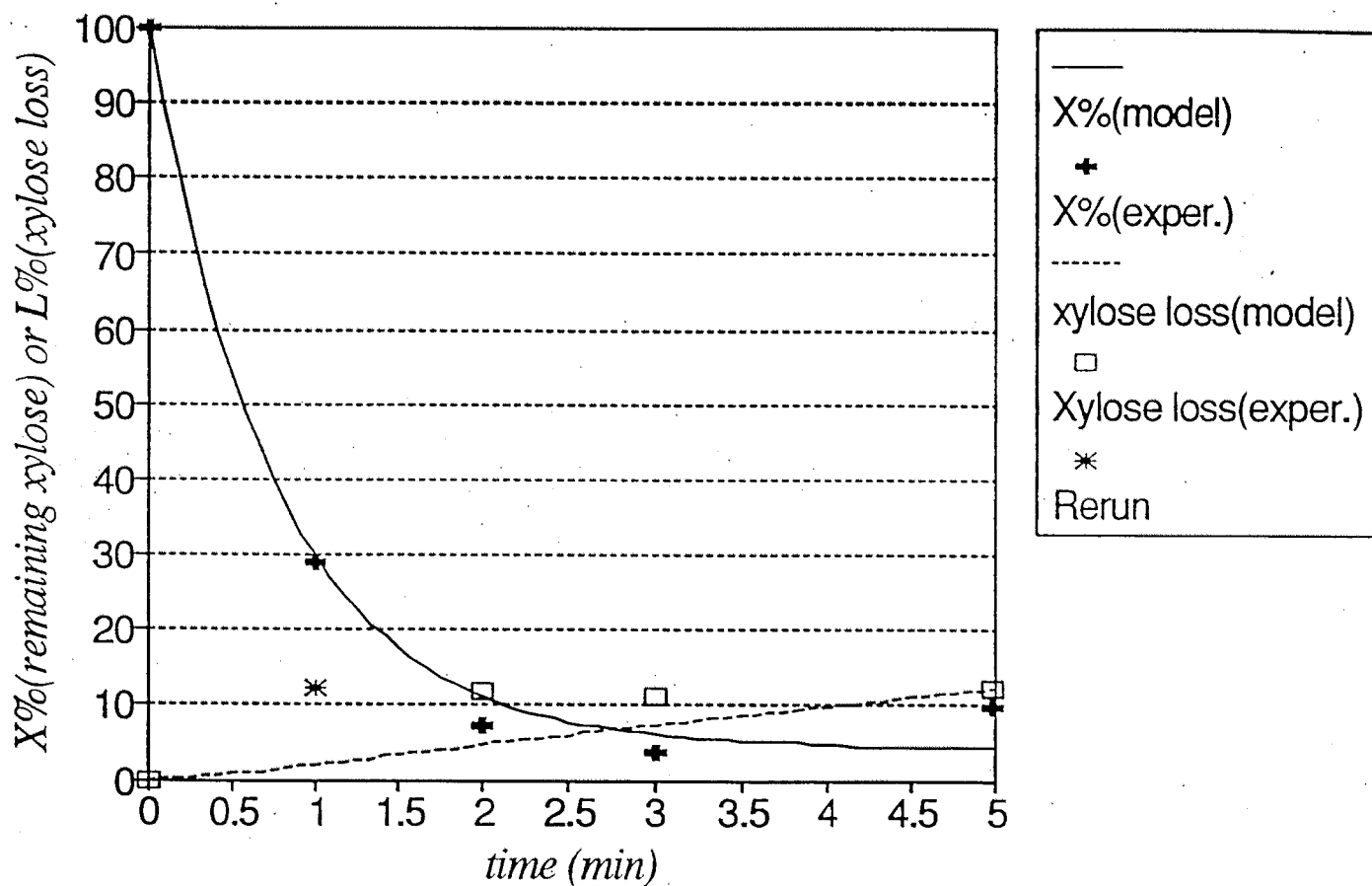
Poplar
160 C, 0.6%



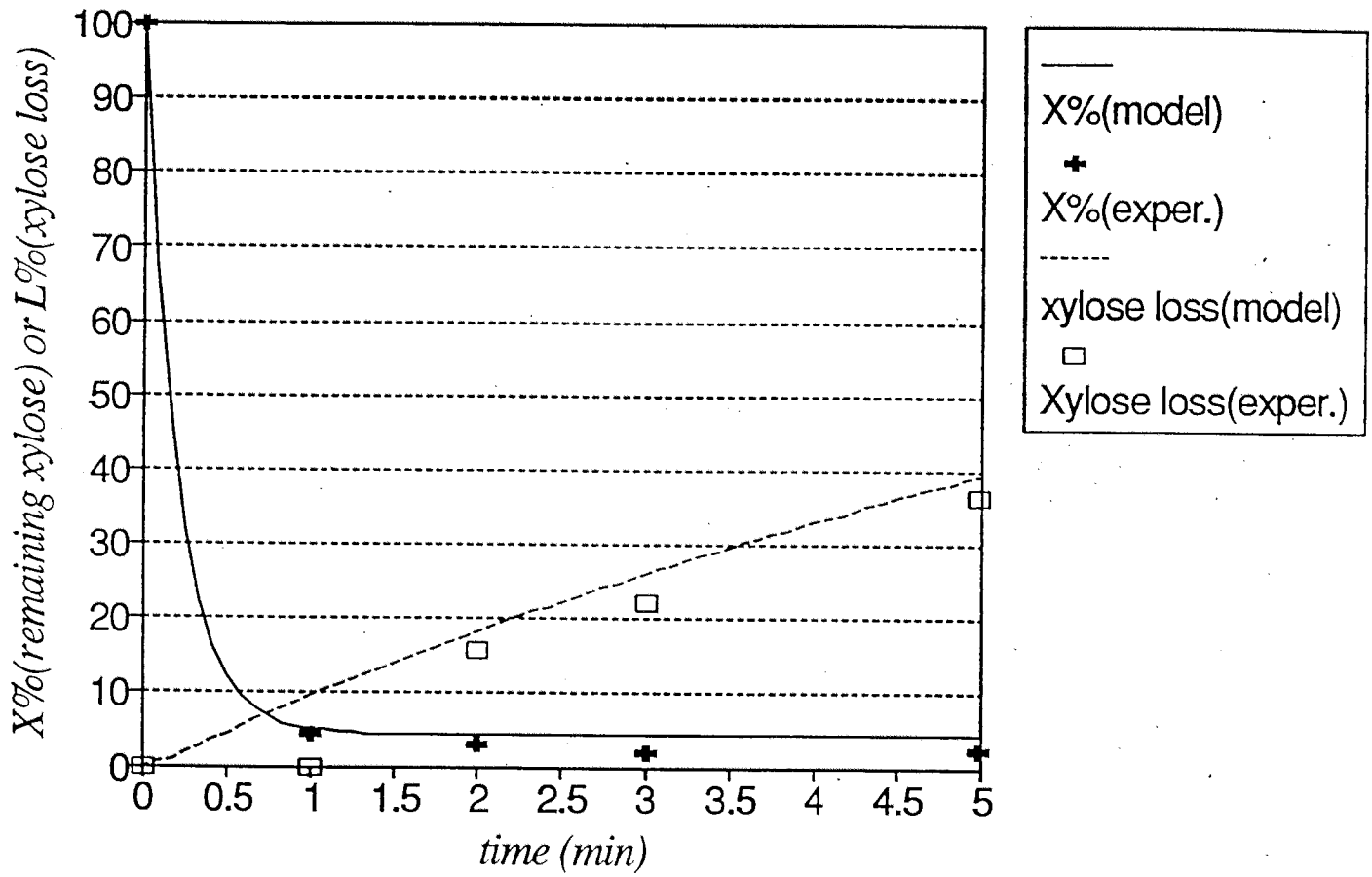
Poplar
160 C, 0.9%



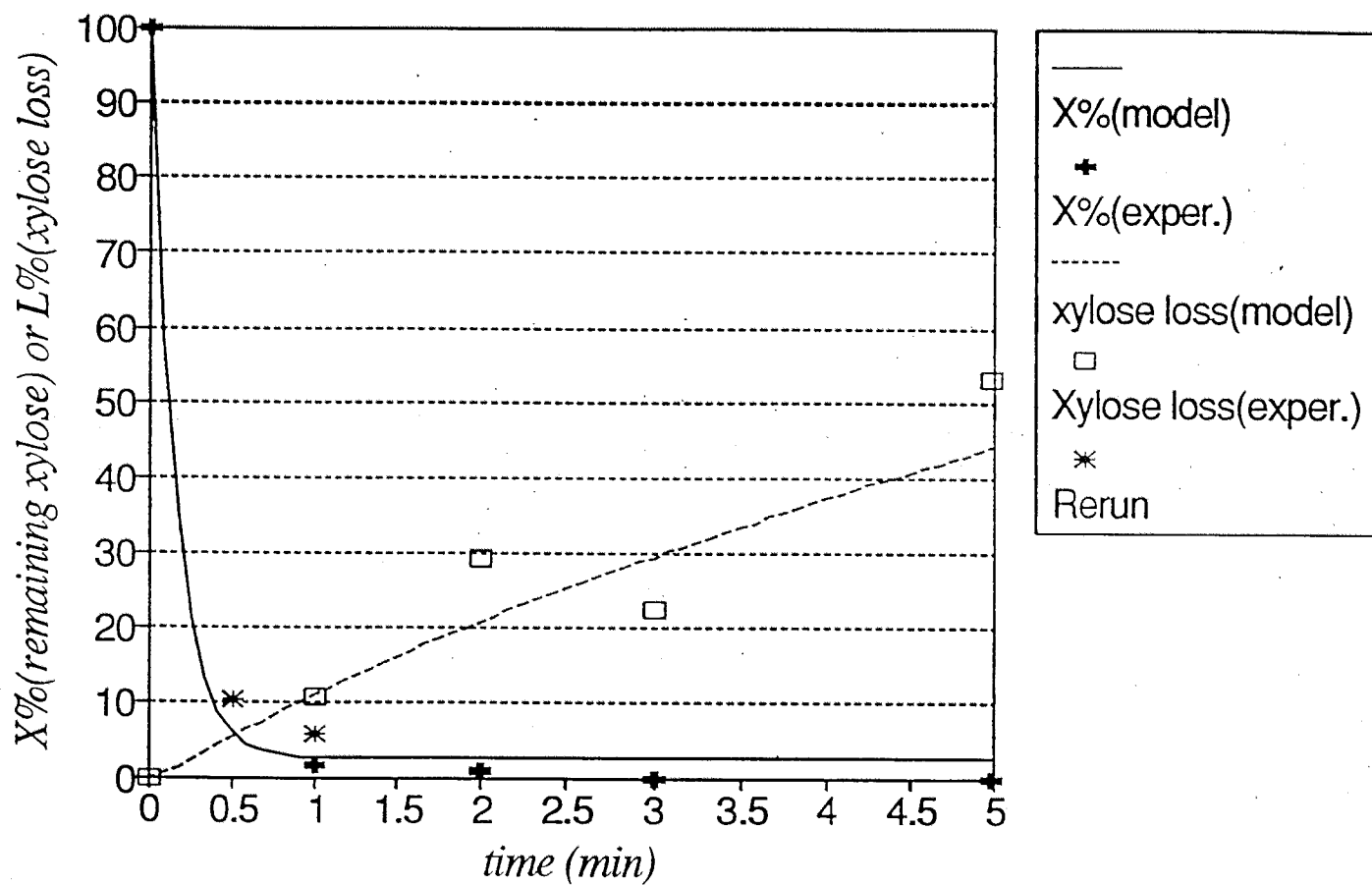
Poplar
160 C, 1.2%



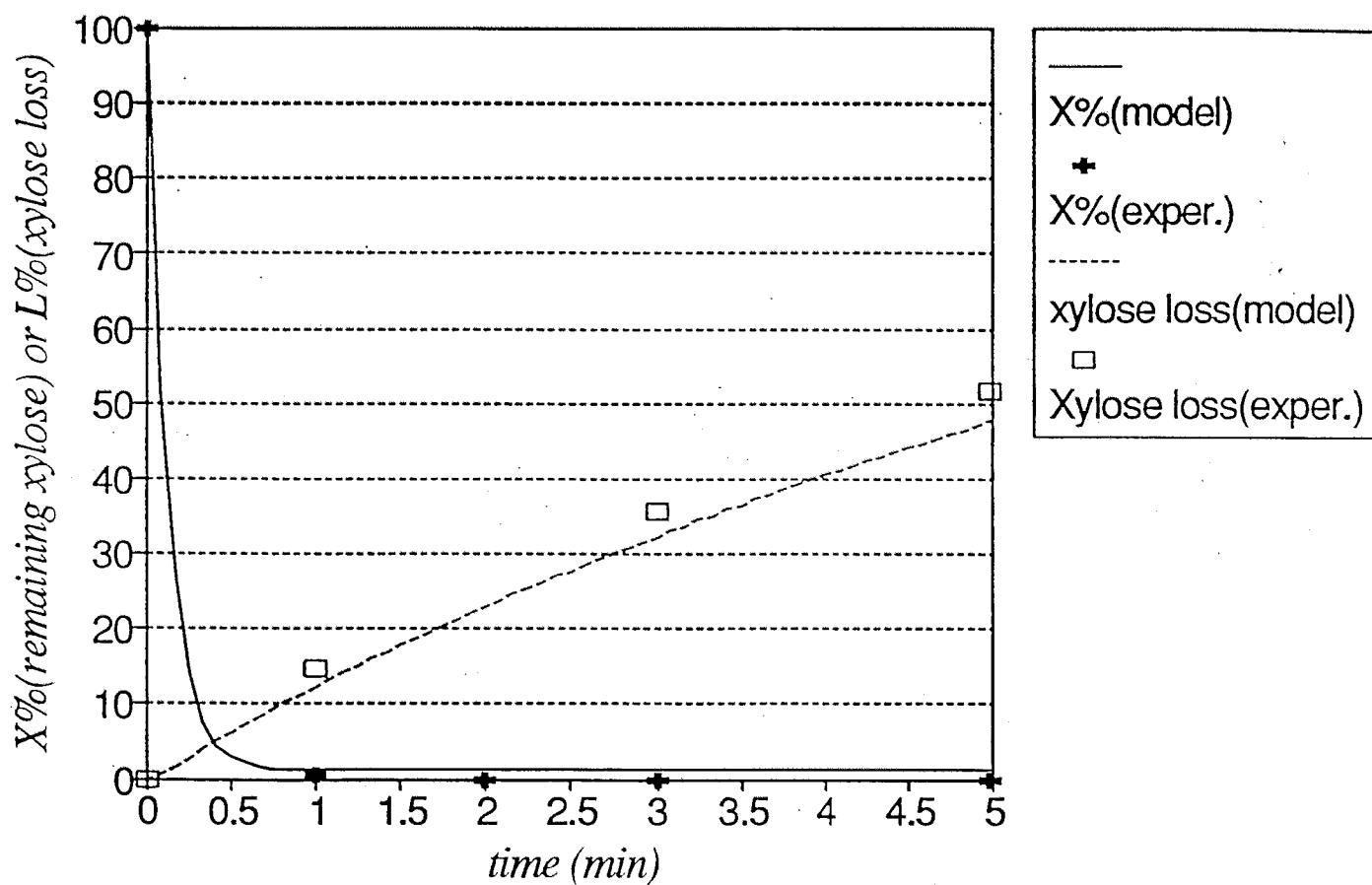
Poplar
180 C, 0.6%



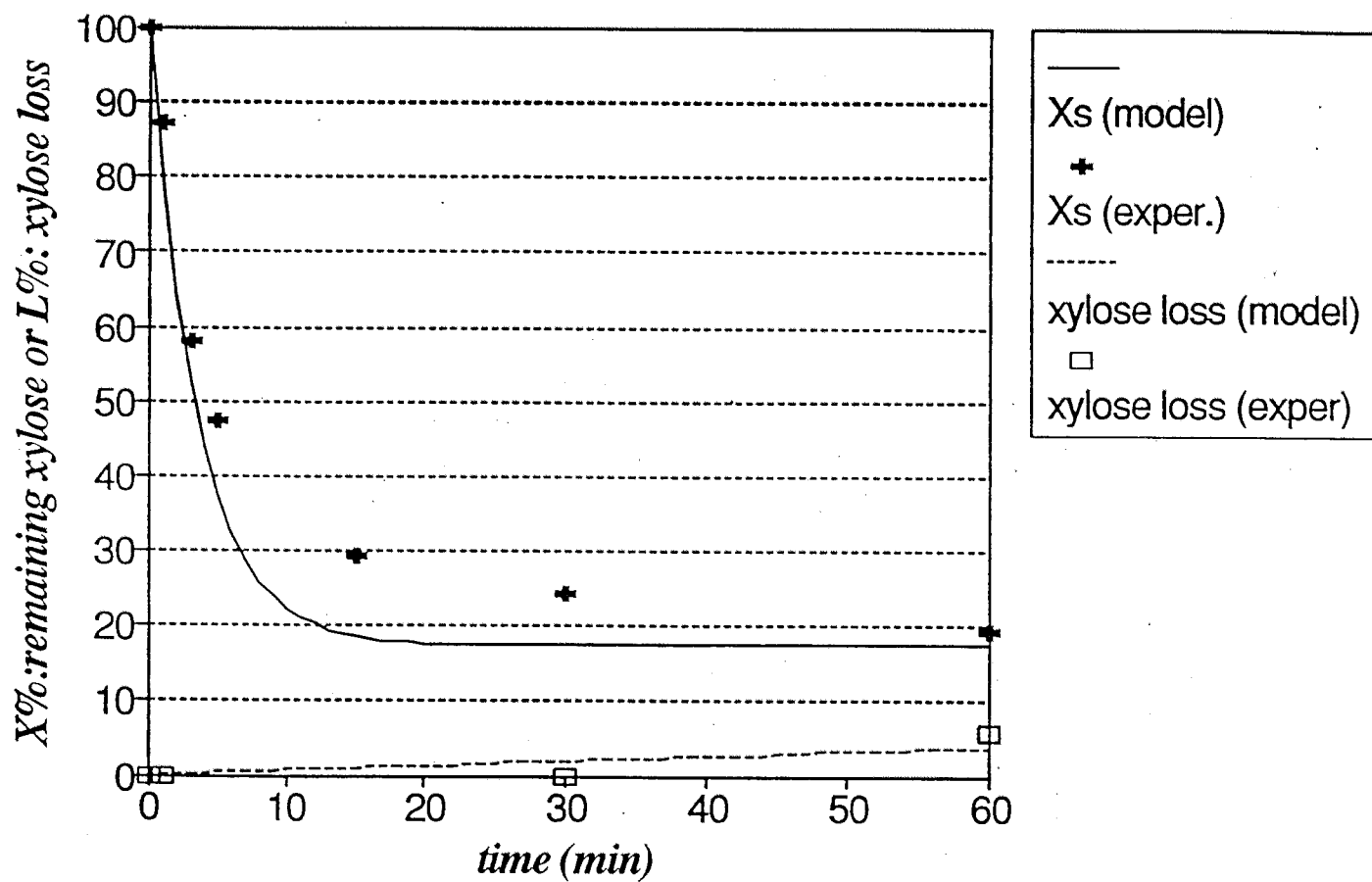
Poplar
180 C, 0.9%



Poplar
180 C, 1.2%

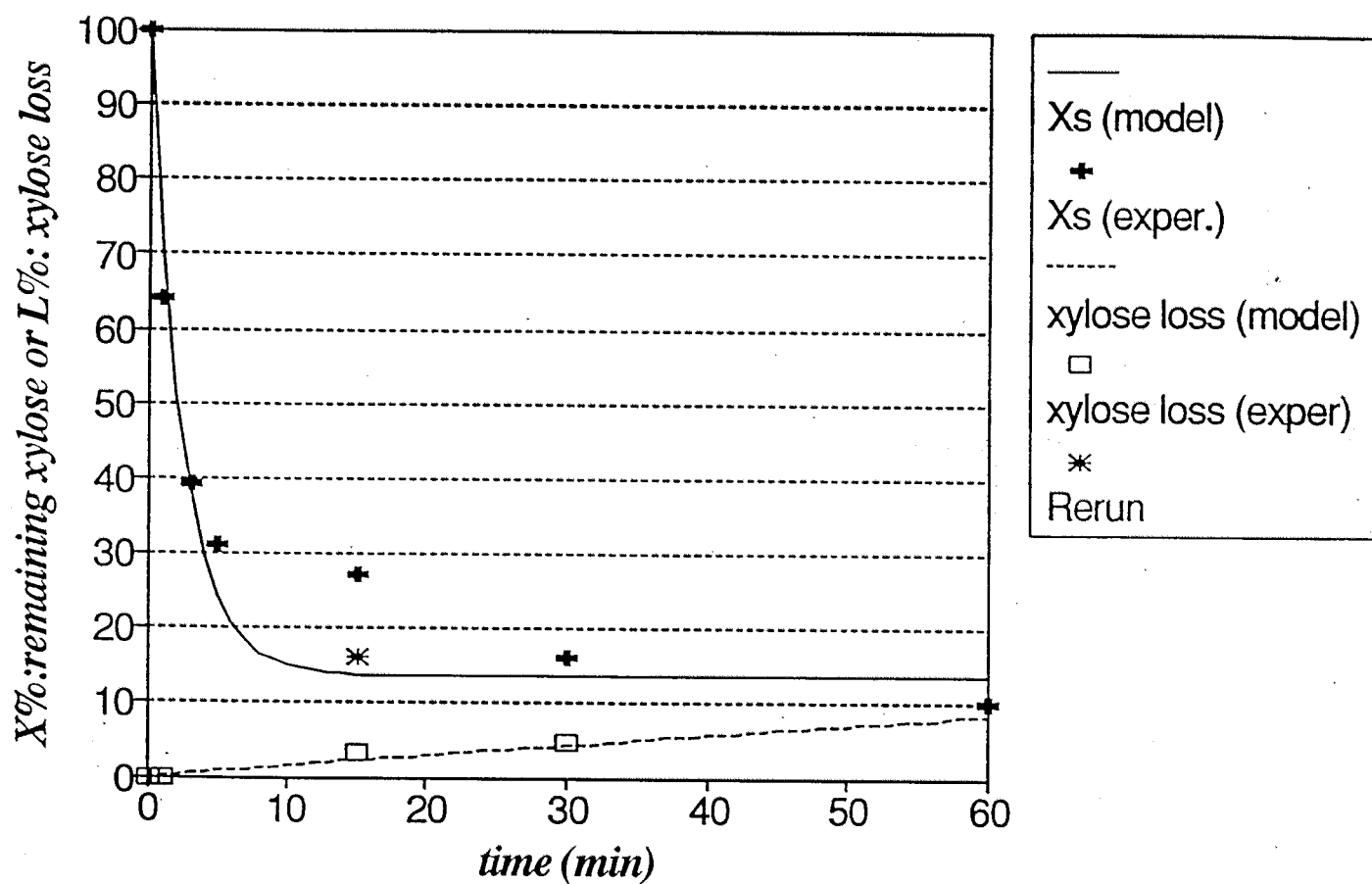


Switchgrass
140 C & 0.6%

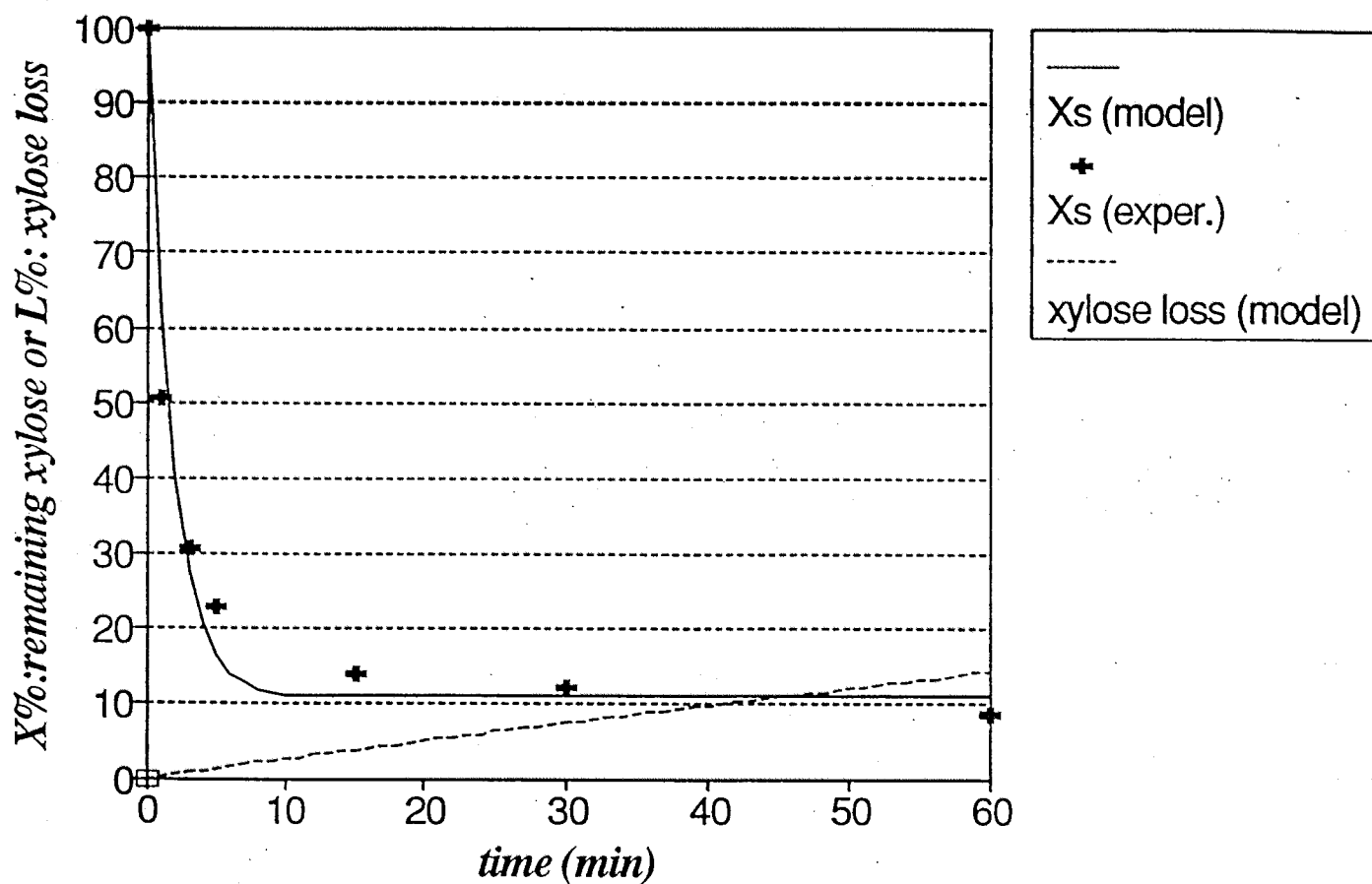


Switchgrass

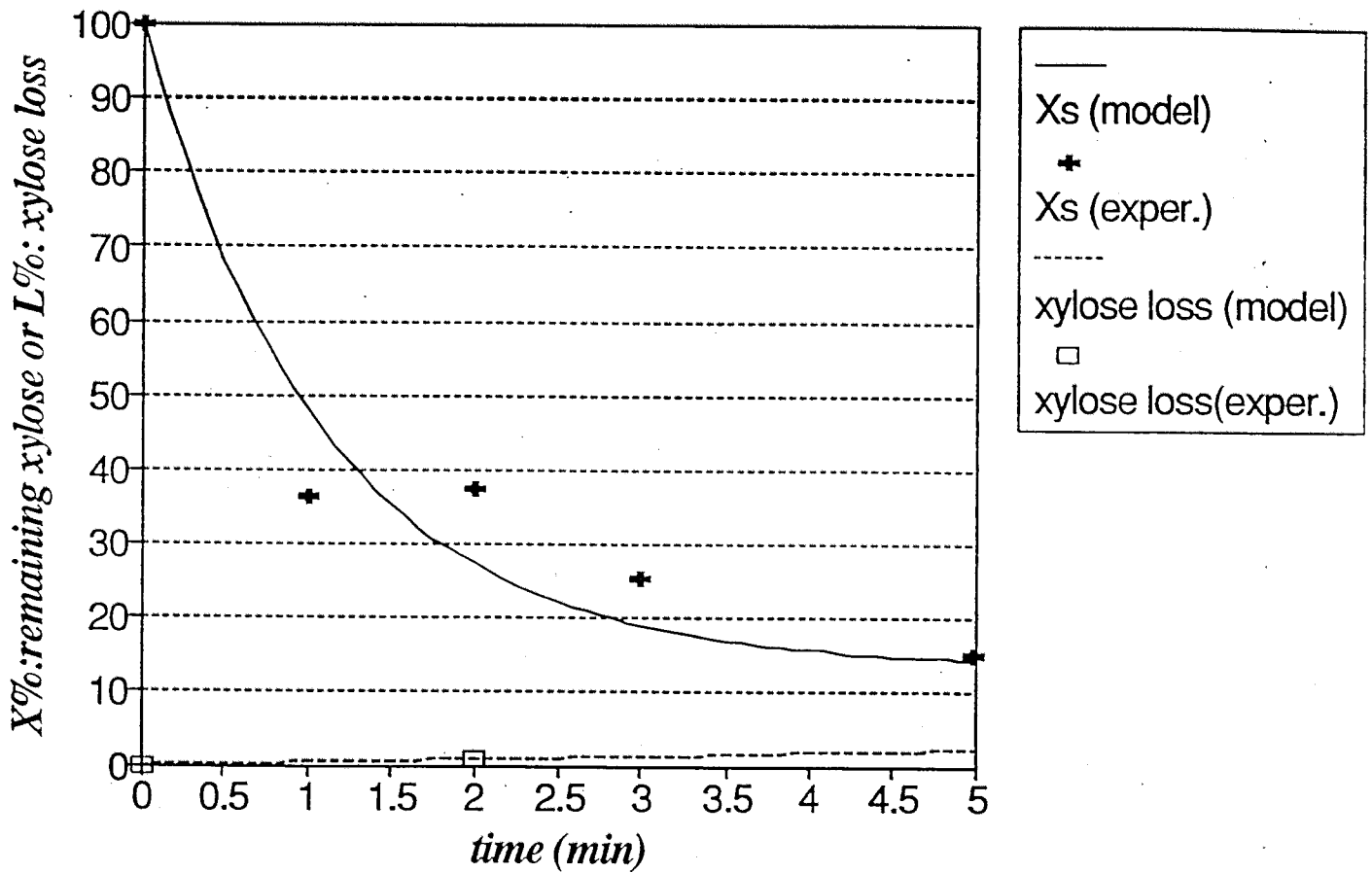
140 C & 0.9%



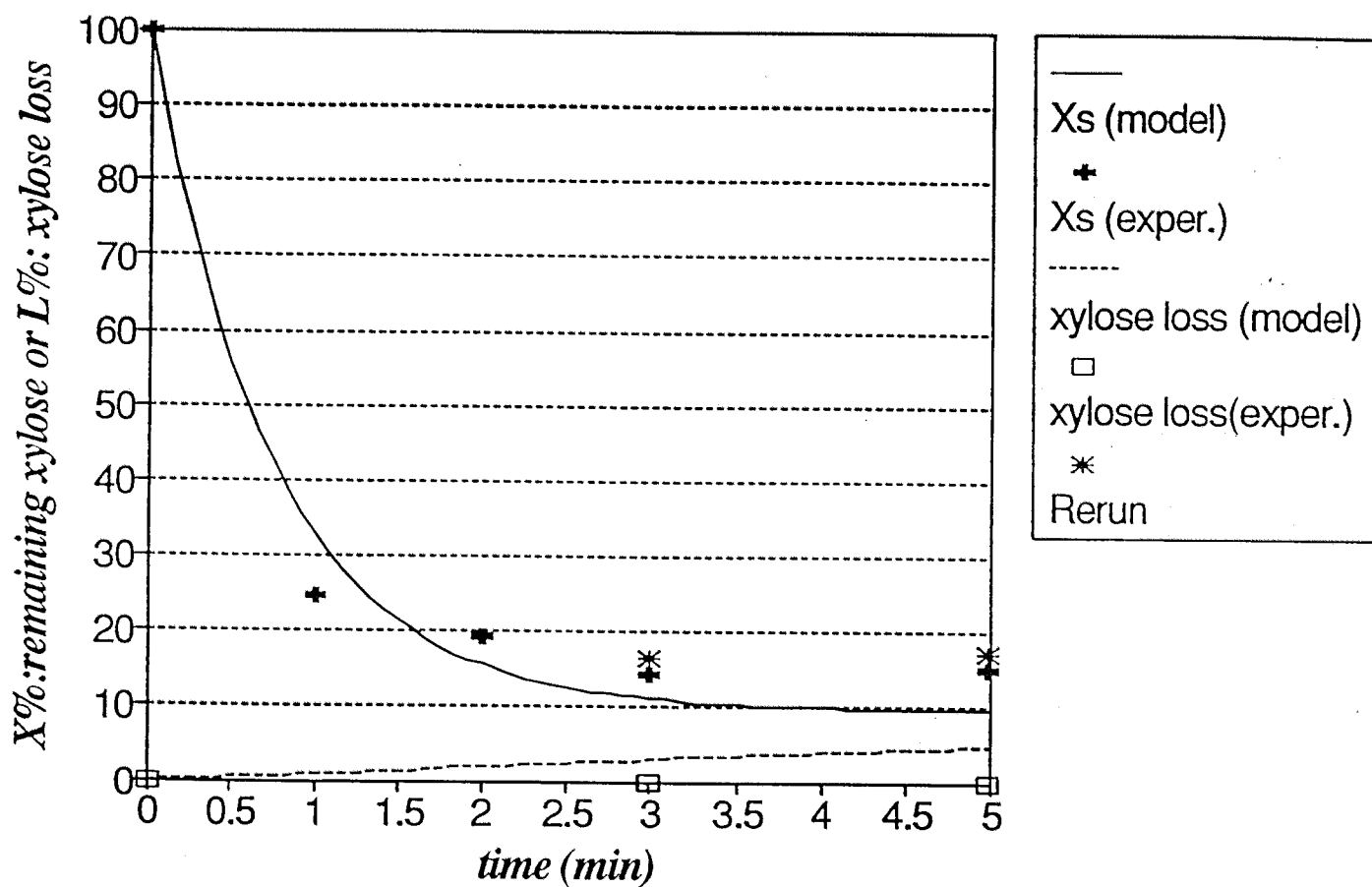
Switchgrass
140 C & 12%



Switchgrass
160 C & 0.6%

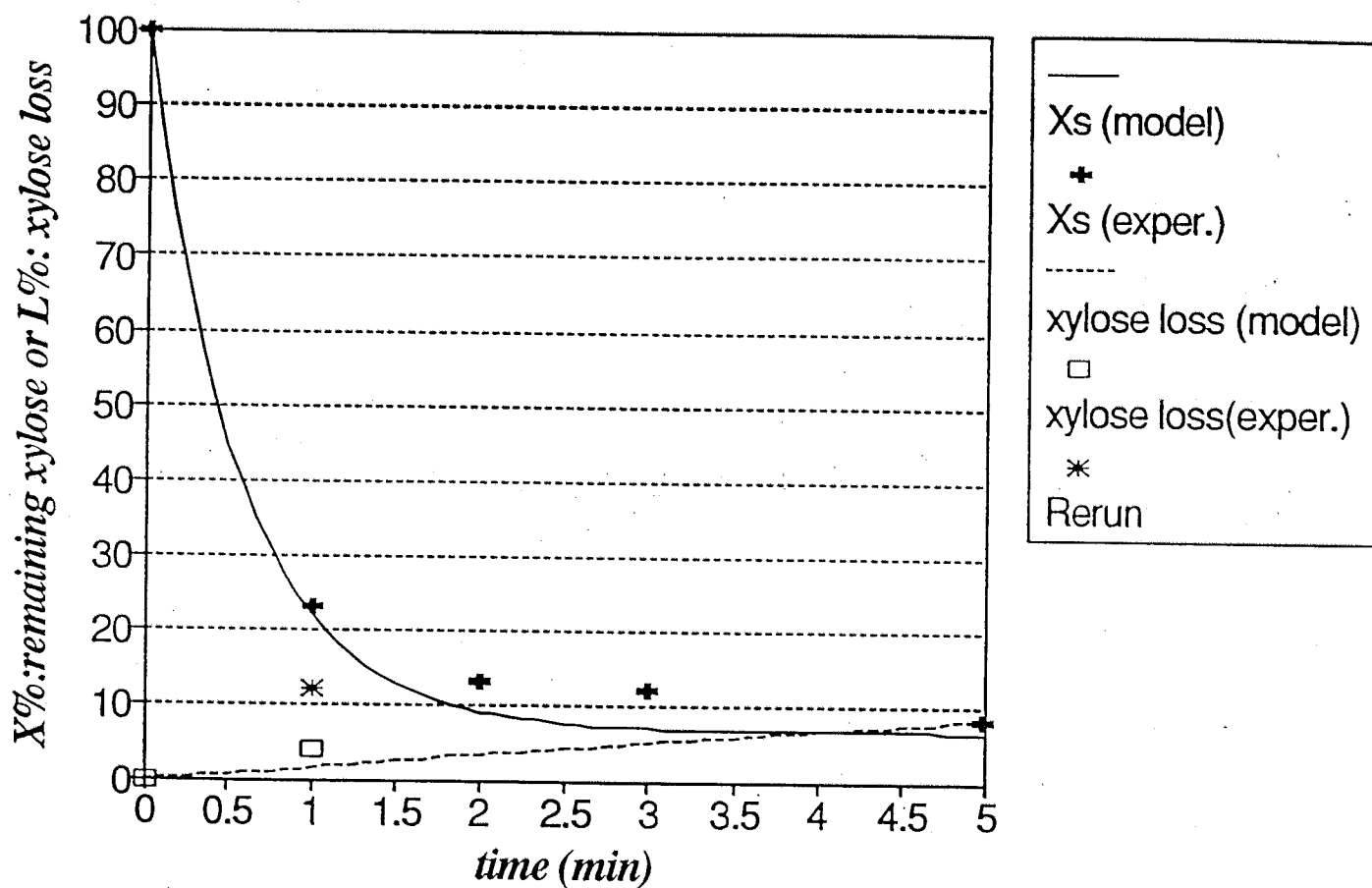


Switchgrass
160 C & 0.9%

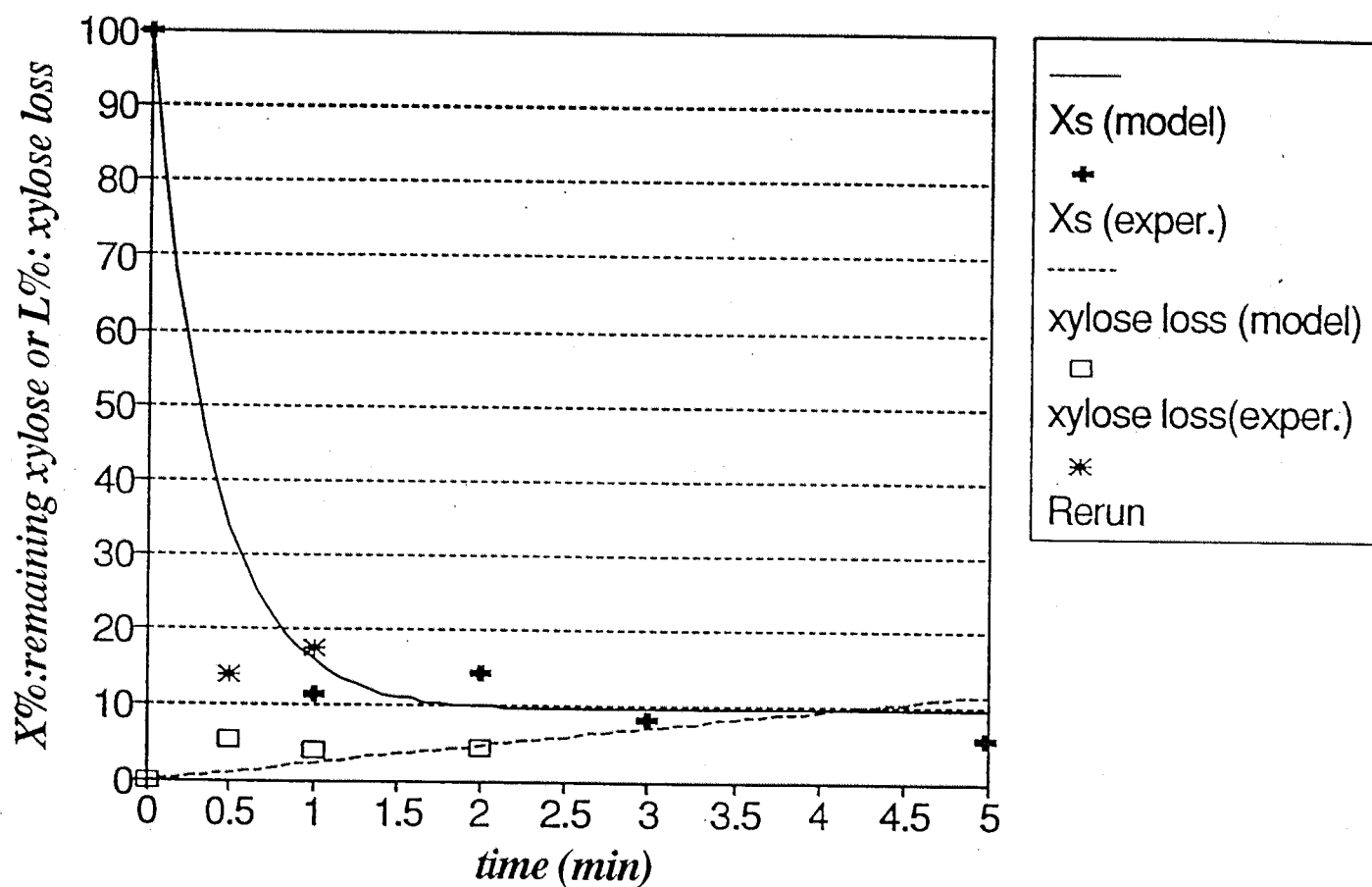


Switchgrass

160 C & 1.2%

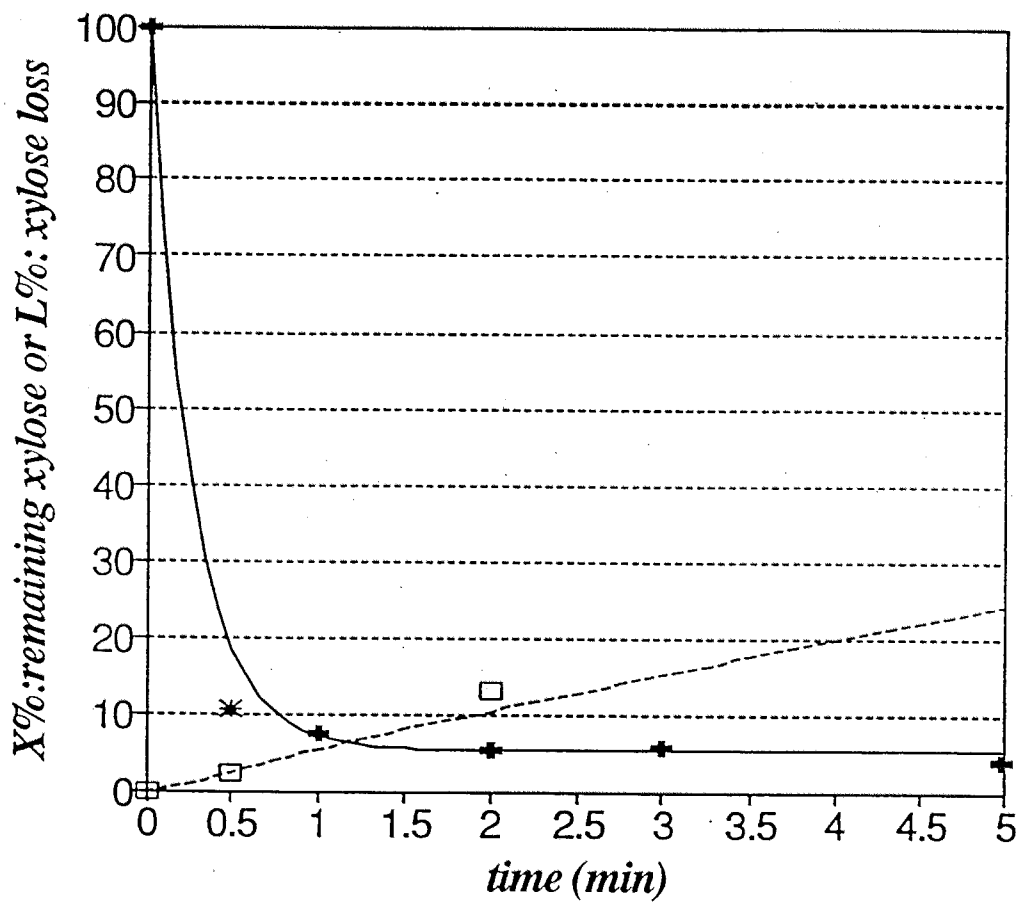


Switchgrass
180 C & 0.6%



Switchgrass

180 C & 0.9%



— Xs (model)

+ Xs (exper.)

- - - xylose loss (model)

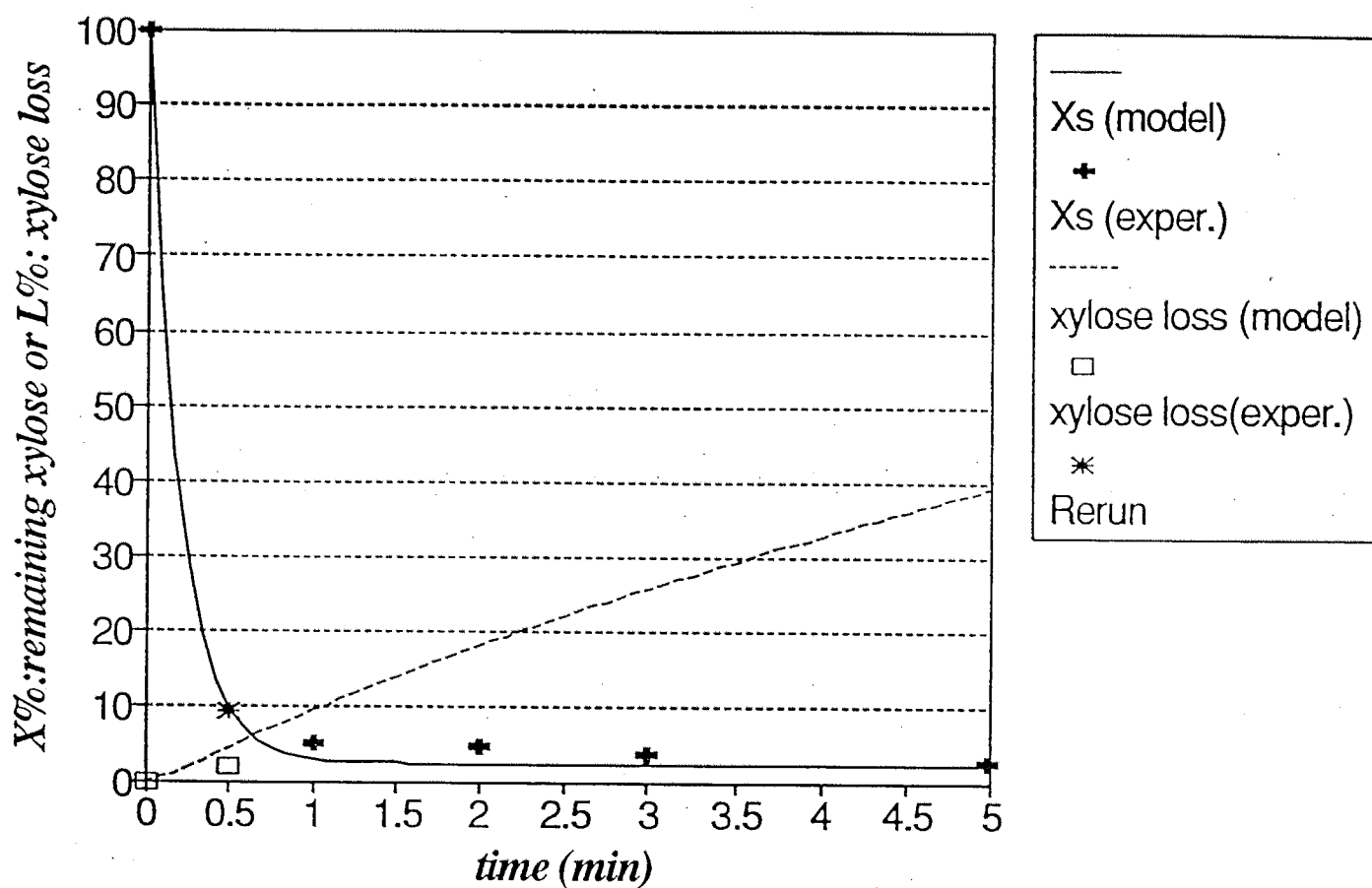
□ xylose loss (exper.)

*

Rerun

Switchgrass

180 C & 1.2%



Appendix 3

The biphasic and single-phase models for predicting the amount of xylose remaining in the pretreated substrates

Pretreatment of each substrate was carried out at nine different combinations of acid concentration and temperature (T & C) with several reaction times. A non-linear regression method was used to fit the experimental values of percentage of xylose remaining in the substrate (X_R) and corresponding time (t) with the biphasic model:

$$X_R = X_f \cdot e^{-k_f t} + X_s \cdot e^{-k_s t}$$

For each of those nine sets, the values of X_f , k_f , and k_s which minimized the objective function (sum of squared errors between the experimental values and those predicted by the biphasic model) were found. These values did not follow a predictable trend with respect to temperature or acid concentration (see attached graphs). For instance, the percentage of xylose remaining in the pretreated substrate (X_f) varied in a relatively wide range. It ranged from 69% to 97% for poplar, and from 51% to 94% for switchgrass. Because of such a wide range, it did not seem reasonable to consider a constant X_f for all reaction conditions. On the other hand, it was not possible to model this parameter with respect to any of the reaction conditions, because there was no consistent relationship between X_f and acid concentration or temperature. In order to

estimate the kinetic constants of the model, however, the average of all X_f values was used for further calculations.

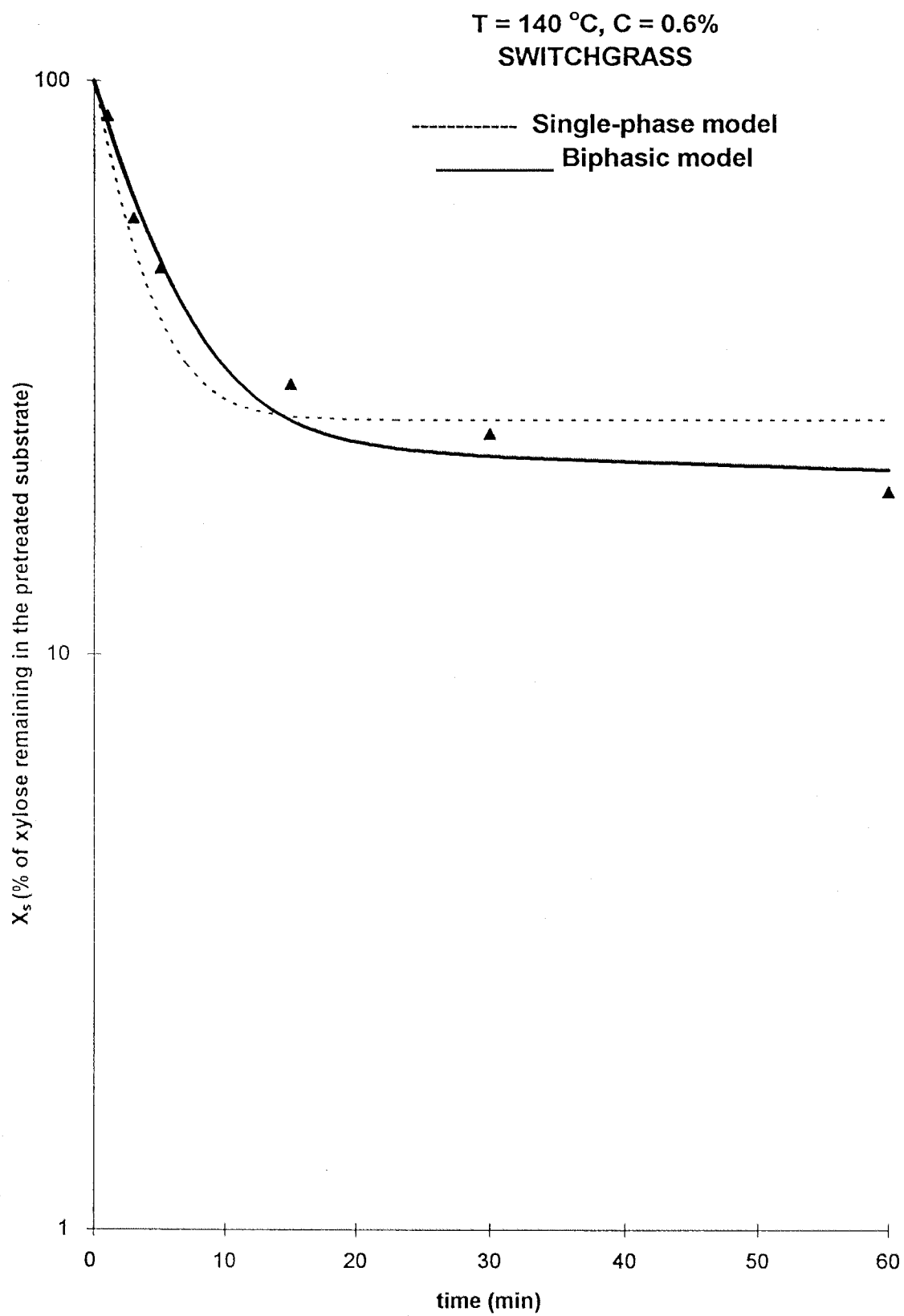
It is worth noting that Grohmann ⁽¹⁾ et al. (1985) have also found a wide range of X_f for wheat straw (47% to 83%), but not for Aspen wood (71% to 80%). Moreover, Maloney ⁽²⁾ et al. (1985) also reported a range of (59.5% to 72.4%) for X_f of paper birch, but did not find a predictable dependency between X_f and reaction conditions (T or C), and used the average value of X_f for all reaction conditions.

The single-phase (single-rate) model assumed that in each substrate there is only one type of hydrolyzable xylan, to which the term "single" refers. However, in order to avoid any confusion, the term "single-rate" would be more appropriate. This model with a term for the recalcitrant xylan fraction (I), agreed with our observation that under certain pretreatment conditions (especially milder conditions) a fraction of the hemicellulose was effectively resistant to hydrolysis (I). The percentage of hydrolyzable xylan (S) in this model was dependent upon reaction conditions (T and C), and had a reasonable trend with respect to both reaction conditions, therefore, its dependency on temperature and acid concentration was modeled by the proposed equation.

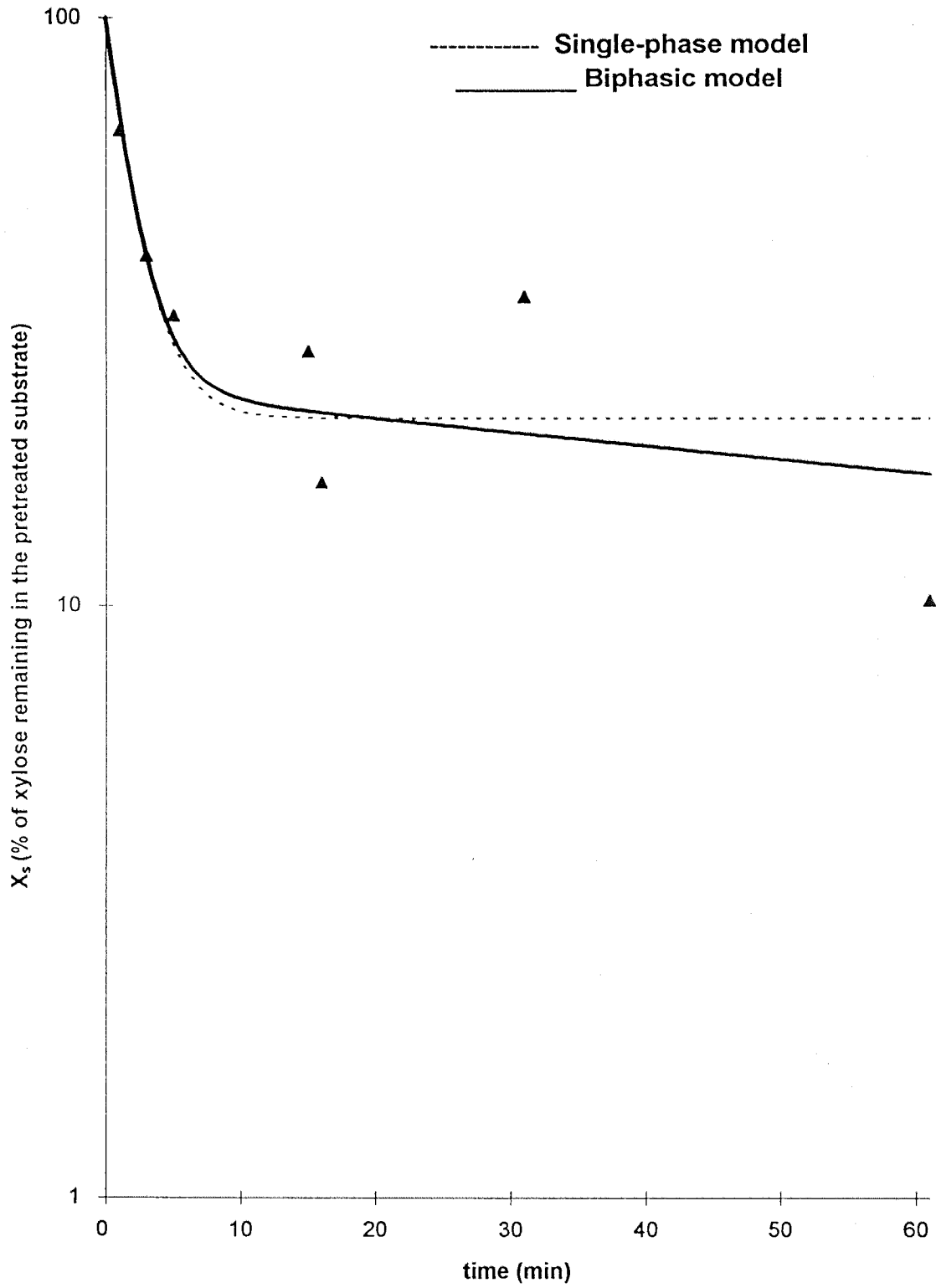
- (1). Grohmann, K., R. Torget and M. Himmel. "Optimization of Dilute Acid Pretreatment of Biomass." Biotechnol. and Bioeng. Symp., No 15. 1985: 59-80
- (2). Maloney, Mark T., Thomas W. Chapman and Andrew J. Baker. "Dilute Acid Hydrolysis of Paper Birch: Kinetics Studies of Xylan and Acetyl Group Hydrolysis." Biotechnol. and Bioeng., 27. 1985: 355-361

In order to see how these two models fit the experimental data, values of xylose remaining in the pretreated substrate predicted by both models as well as experimental data are plotted on a semi-log scale. These graphs indicate that within the range of reaction conditions used for this work both models provide relatively close fit for the experimental data. However, further work is required to study the effect of time on the 'T' (recalcitrant xylan) in the single-rate model, especially at milder conditions. Moreover, at each reaction time more replicates are required to assess the accurate trend of the data.

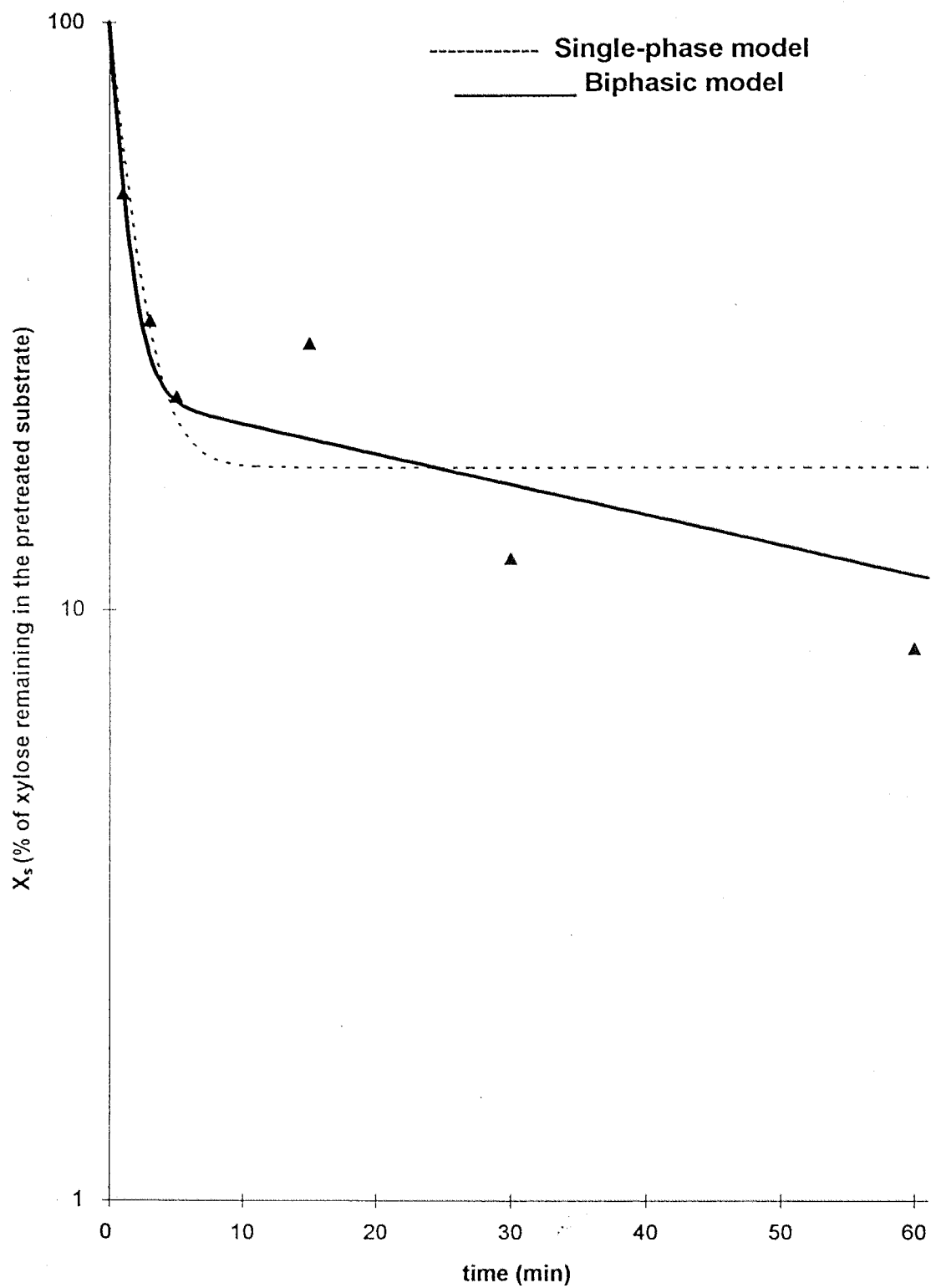
An extensive research is also required to study the effect of reaction conditions on X_f in the biphasic model. For this case, it may seem reasonable to use the average value, or the optimum value of X_f obtained from a statistical analysis, however, for a wider range of reaction conditions (T and C) such a study is necessary.



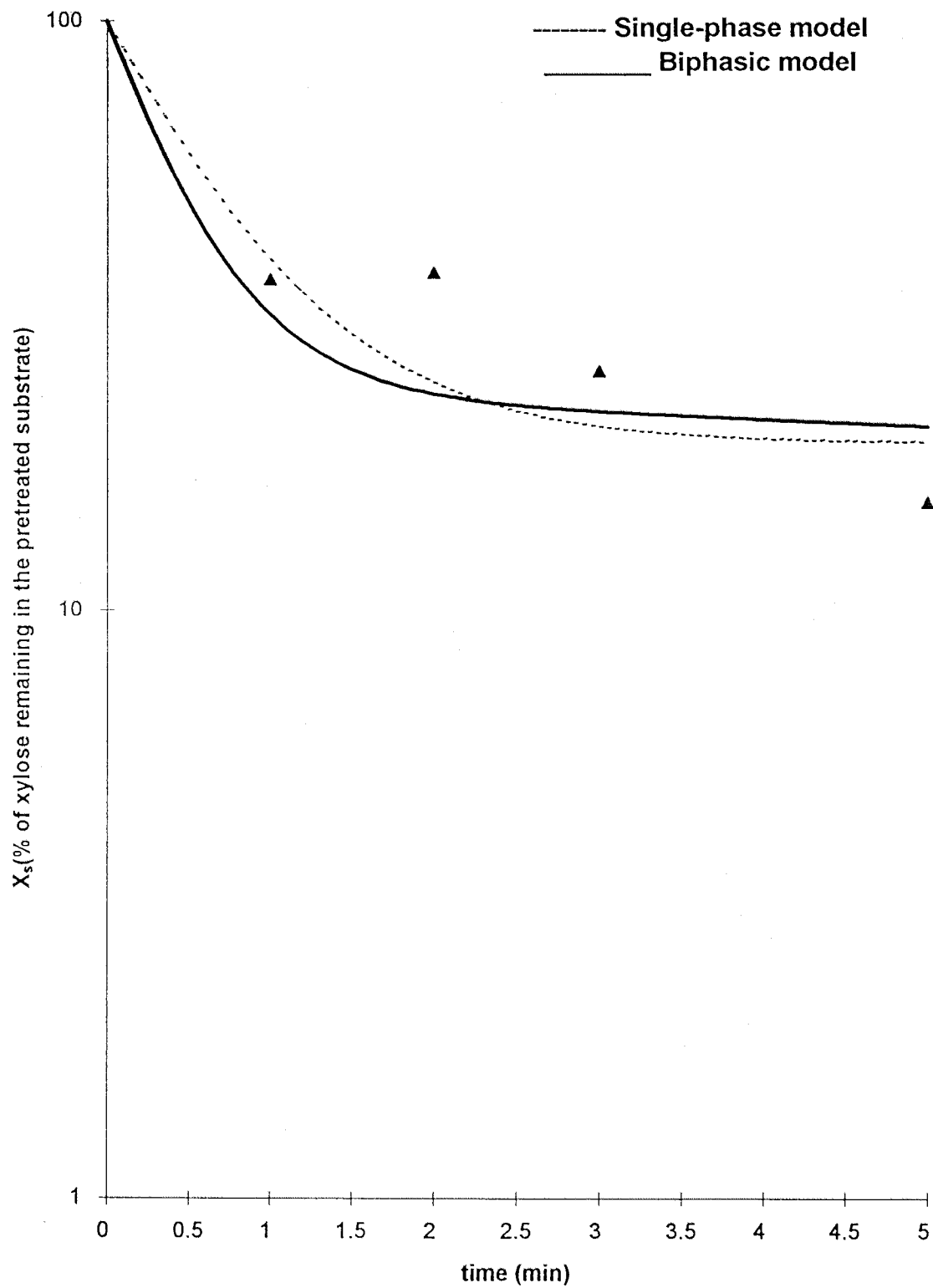
$T = 140\text{ }^{\circ}\text{C}$, $C = 0.9\%$
SWITCHGRASS



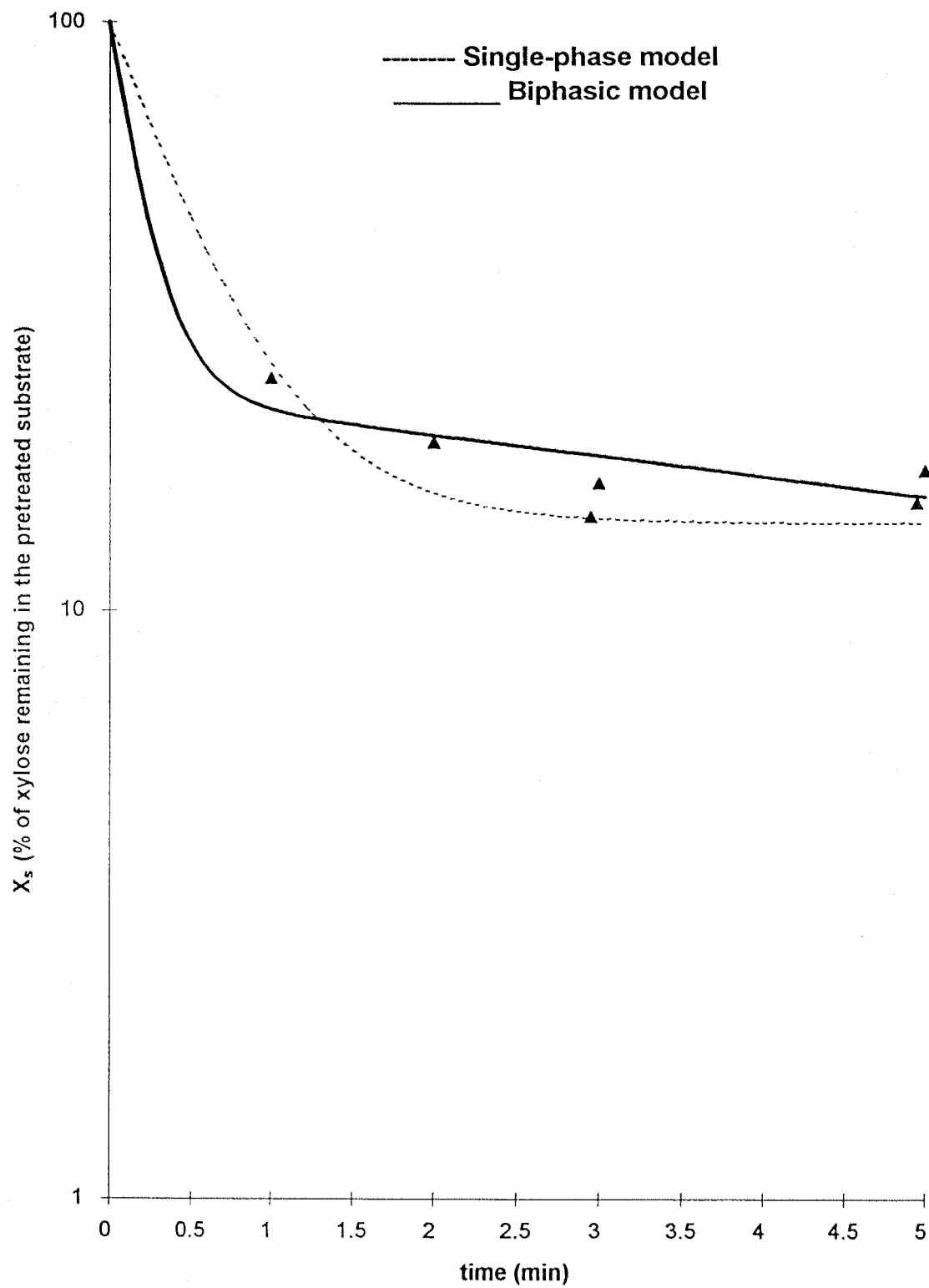
$T = 140\text{ }^{\circ}\text{C}$, $C = 1.2\%$
SWITCHGRASS



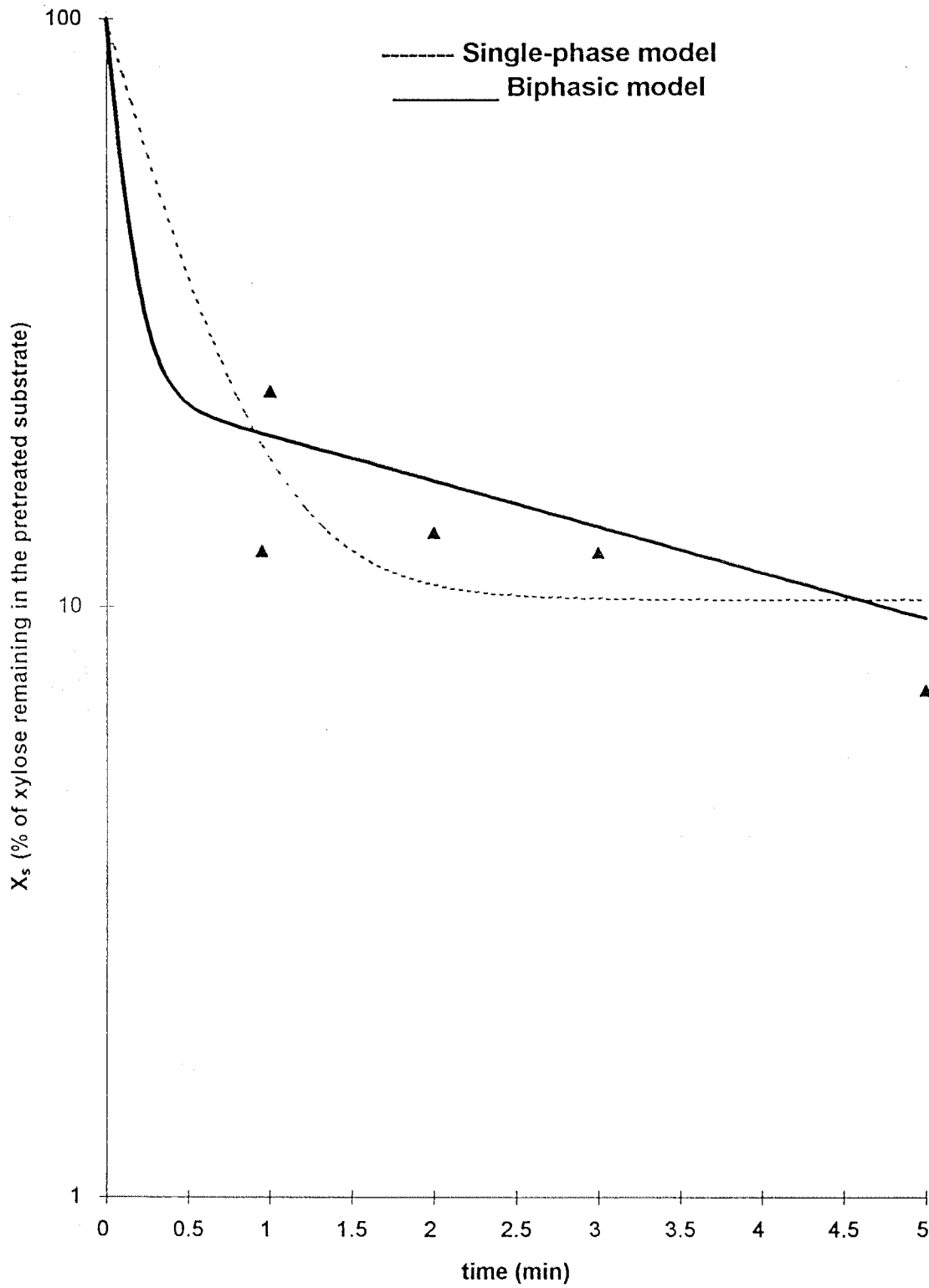
$T = 160\text{ }^{\circ}\text{C}$, $C = 0.6\%$
SWITCHGRASS

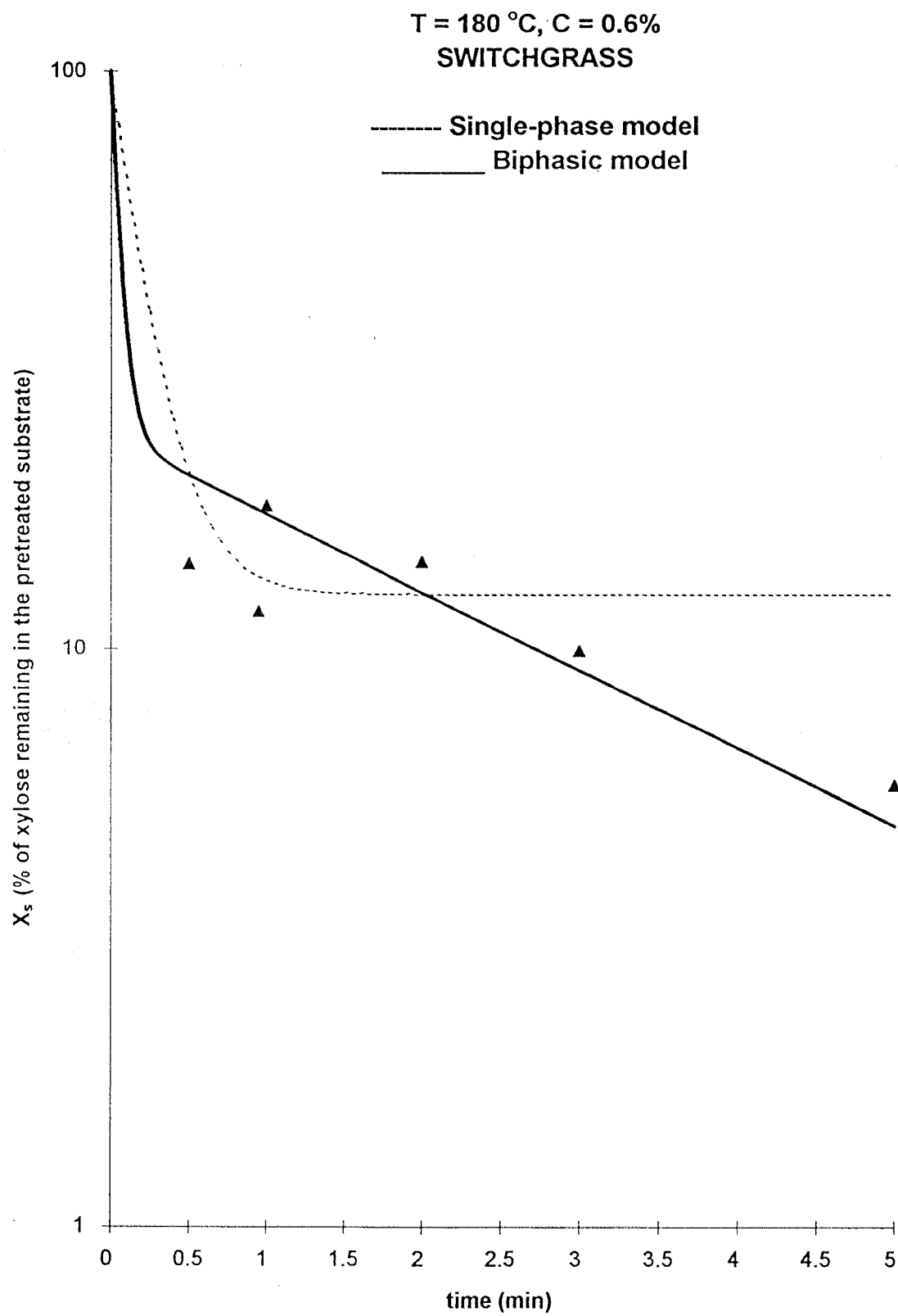


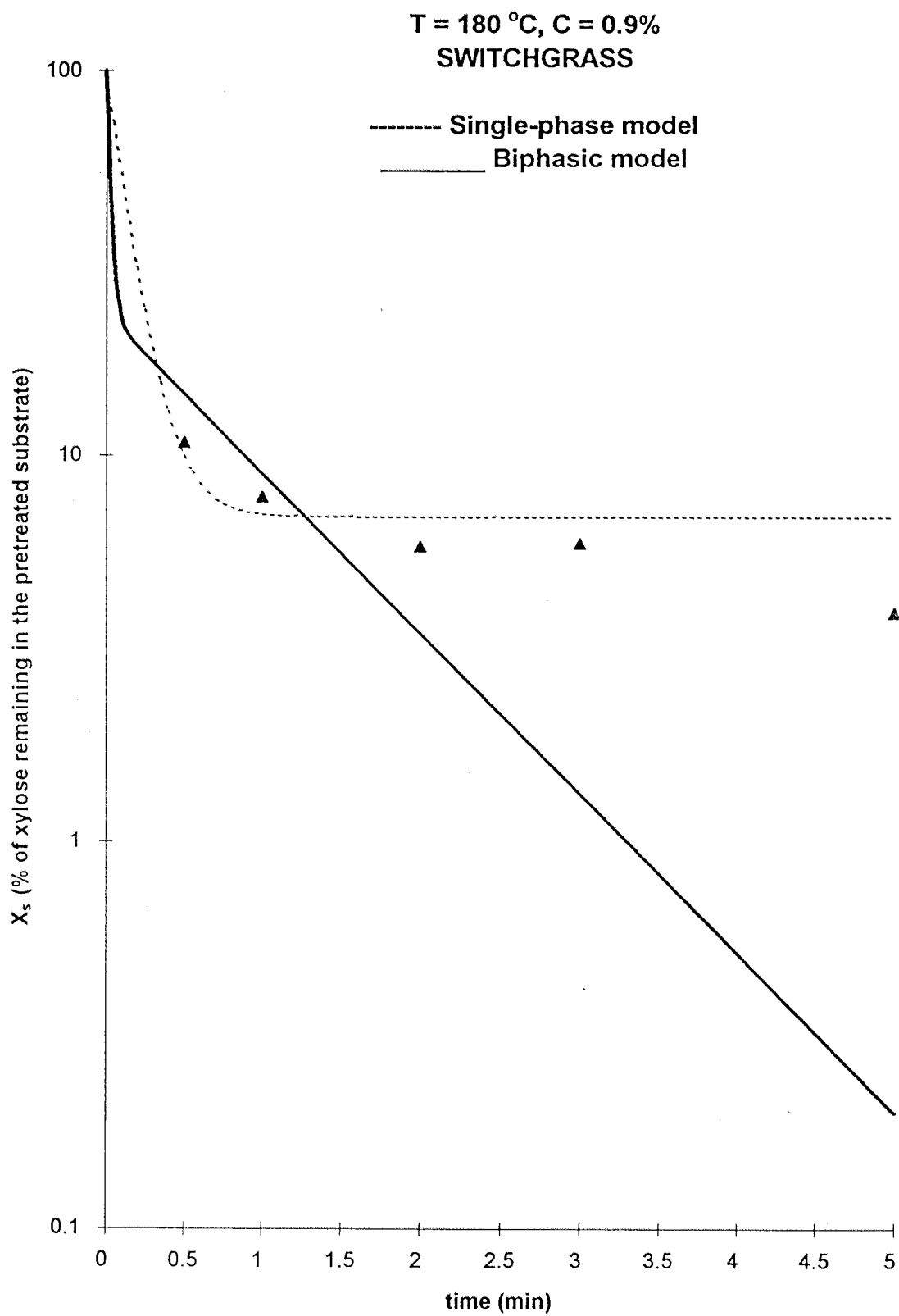
$T = 160\text{ }^{\circ}\text{C}$, $C = 0.9\%$
SWITCHGRASS



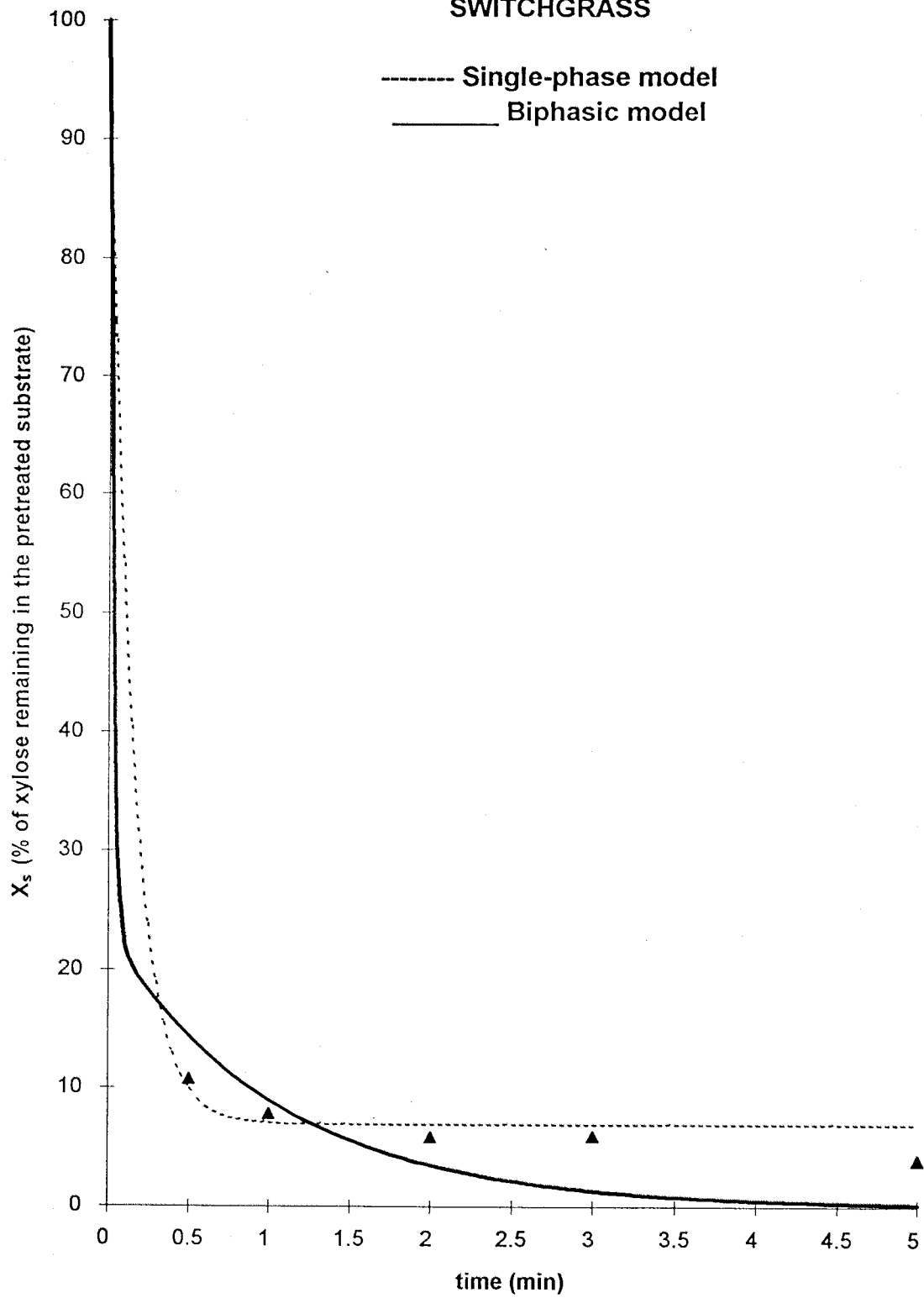
$T = 160\text{ }^{\circ}\text{C}$, $C = 1.2\%$
SWITCHGRASS



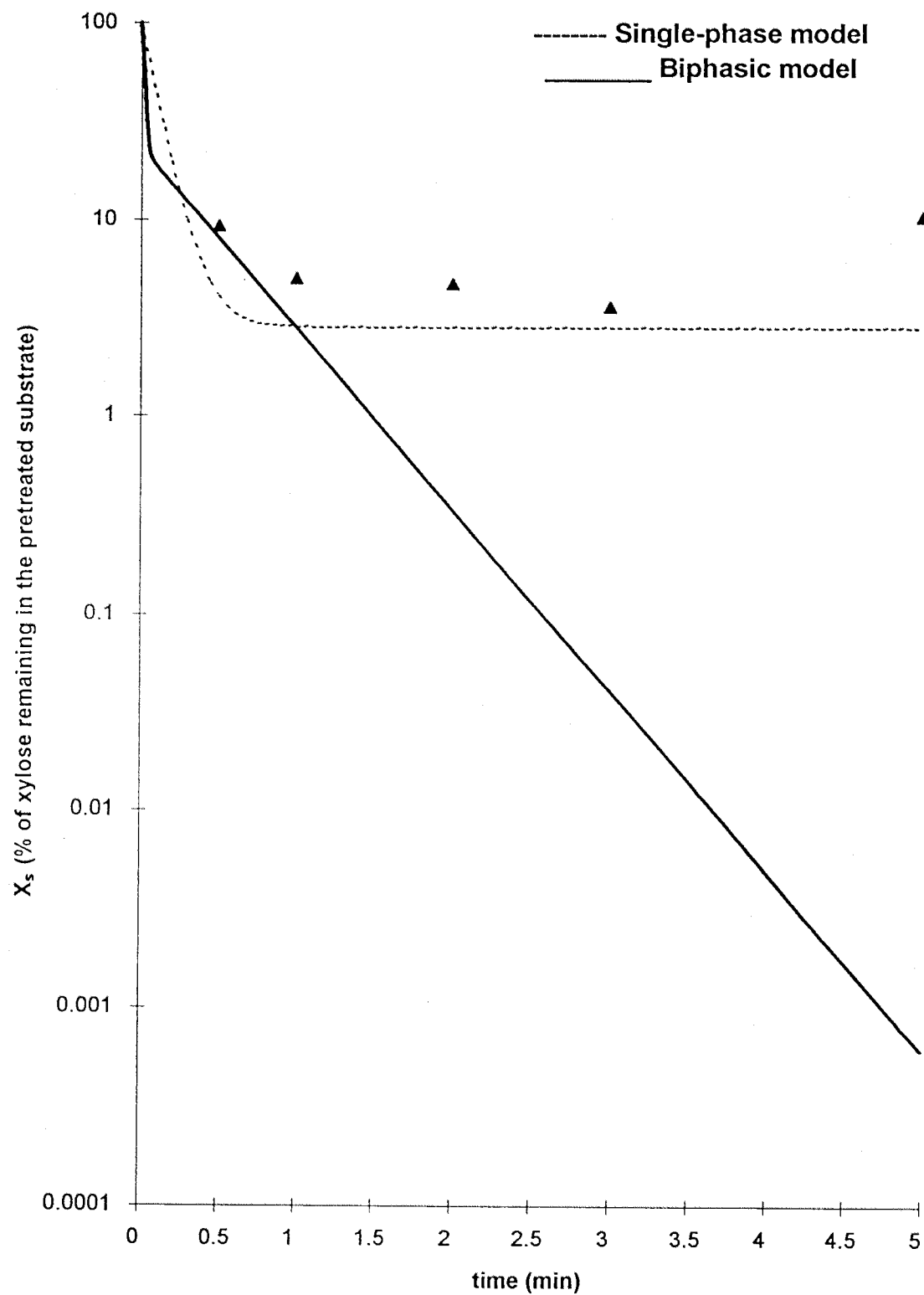




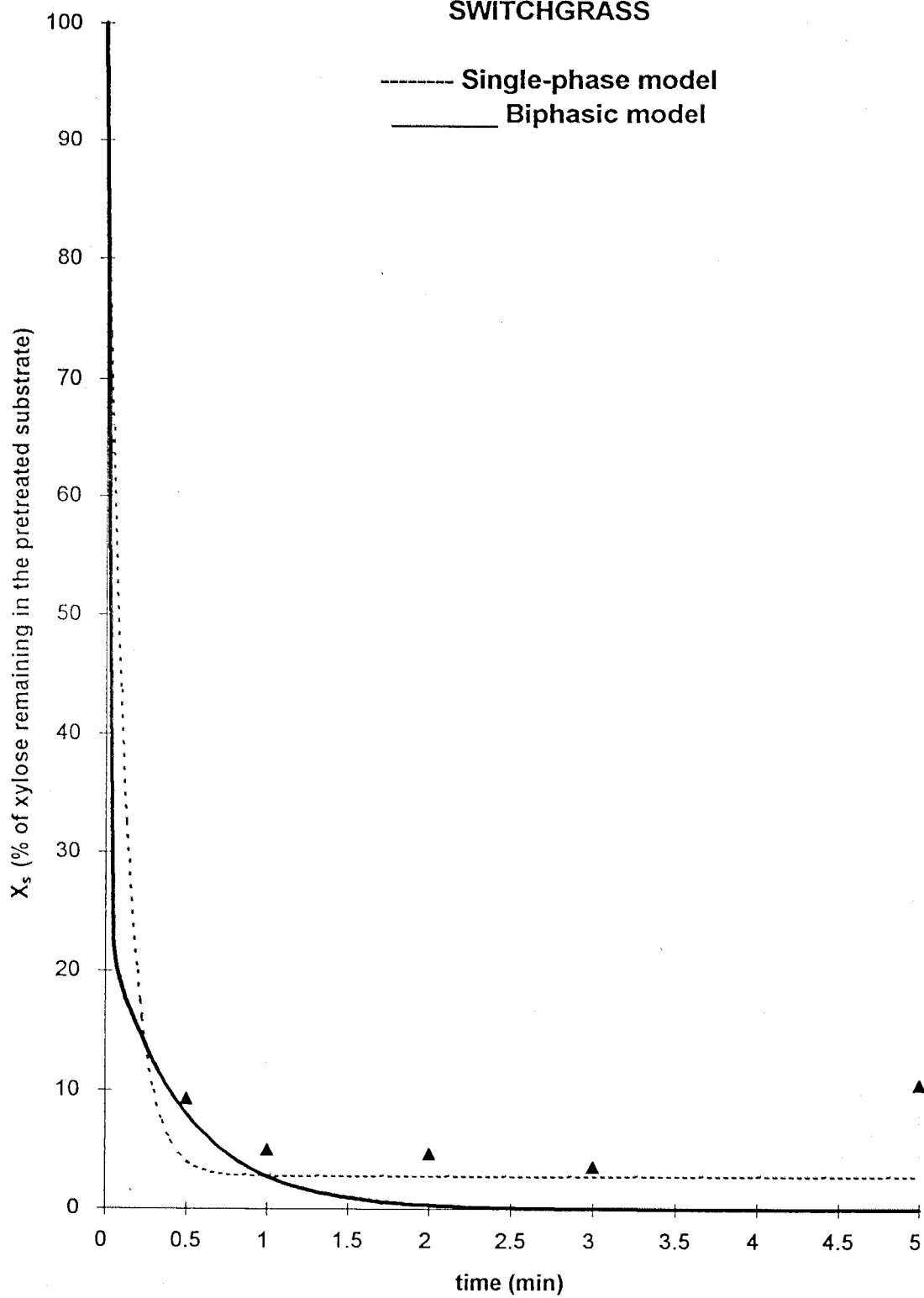
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SWITCHGRASS

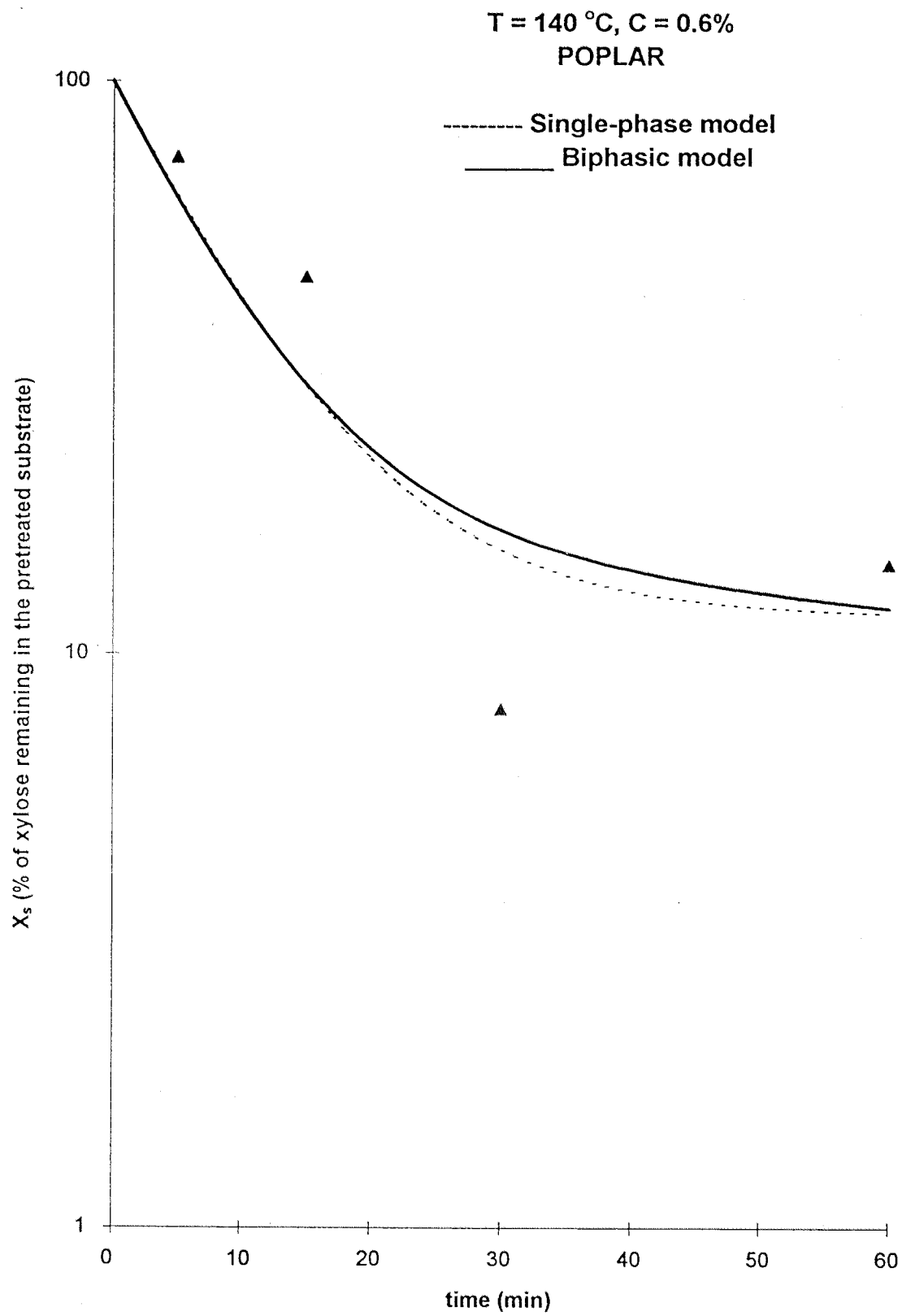


$T = 180\text{ }^{\circ}\text{C}$, $C = 1.2\%$
SWITCHGRASS



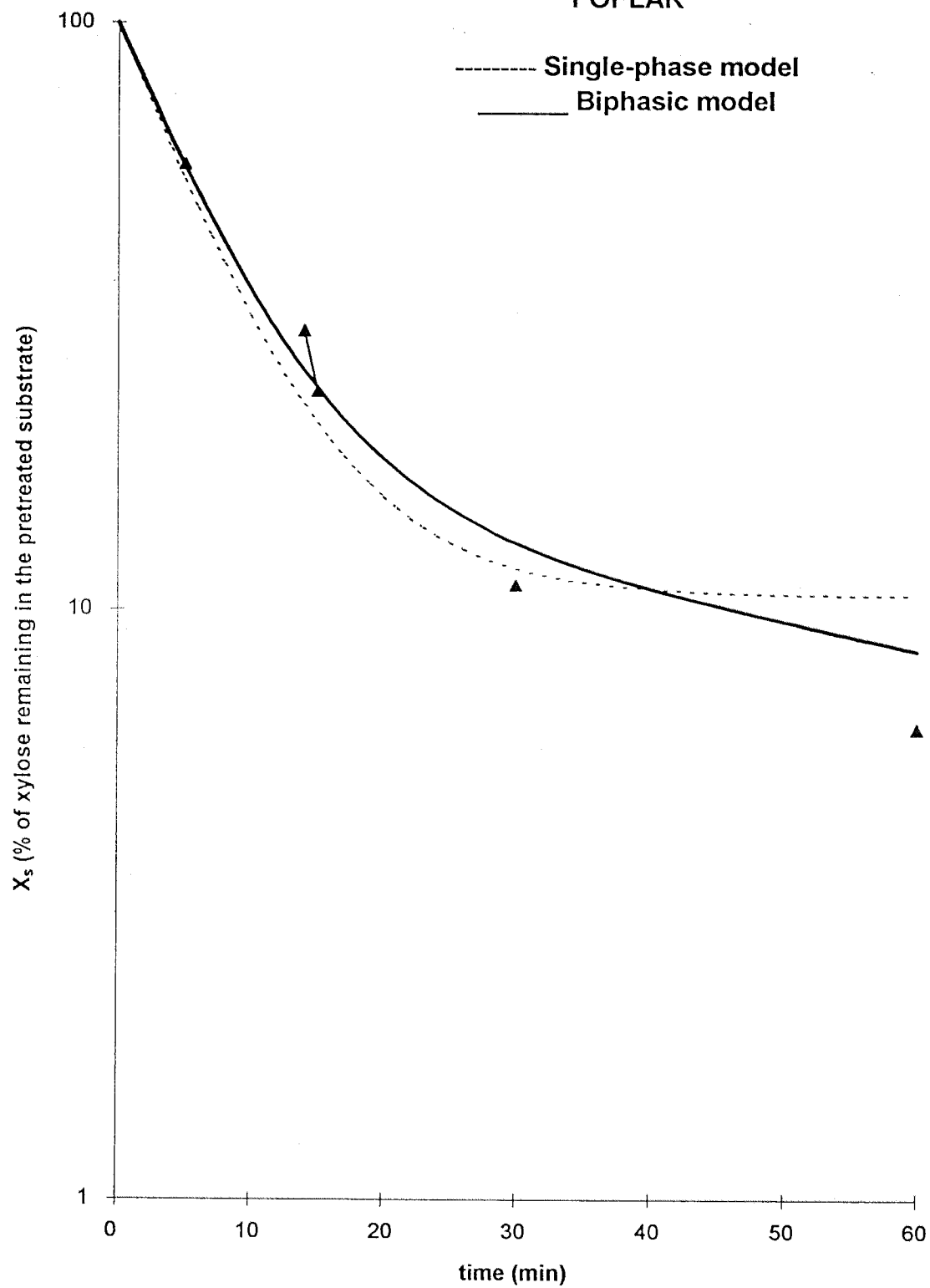
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SWITCHGRASS

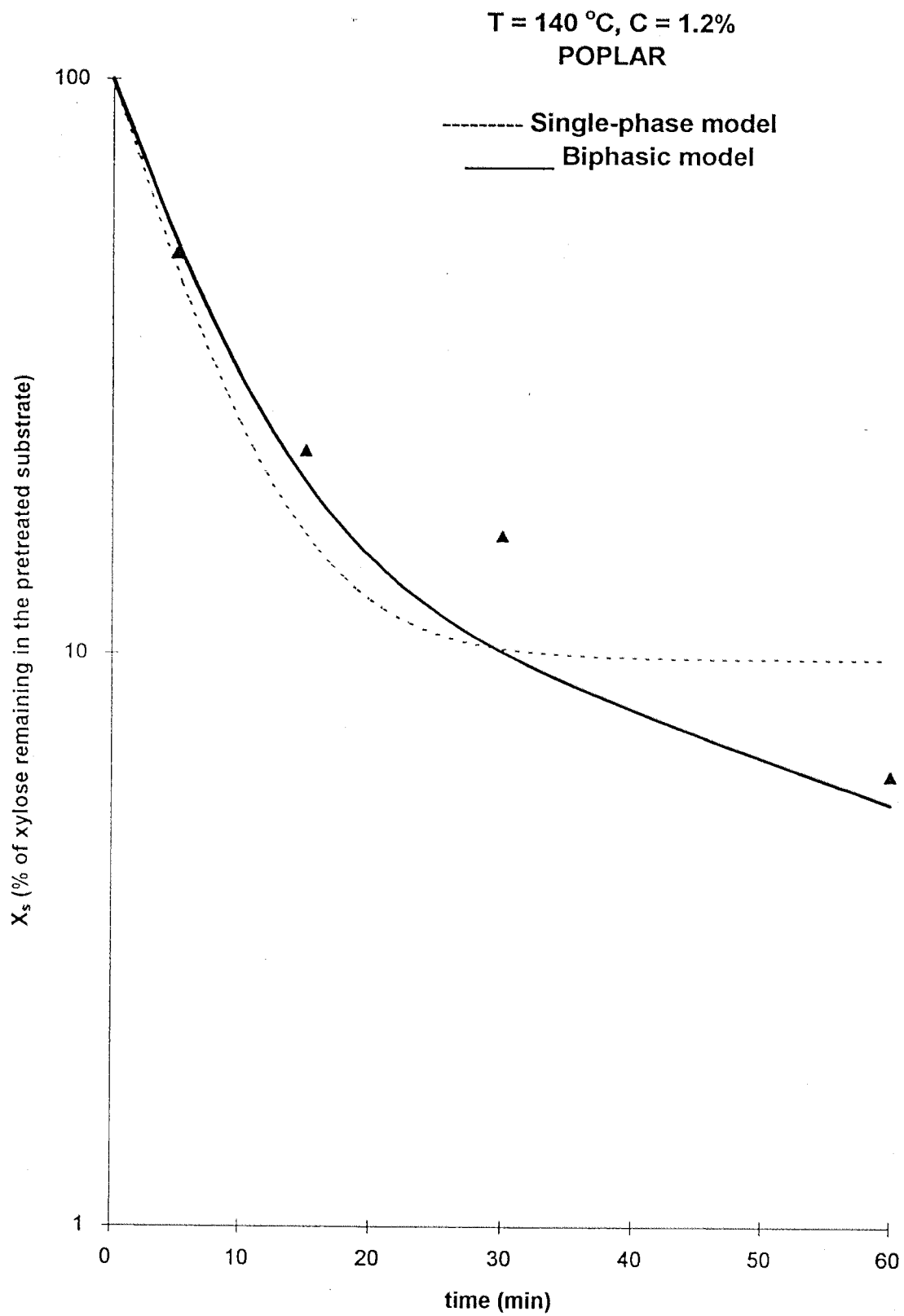


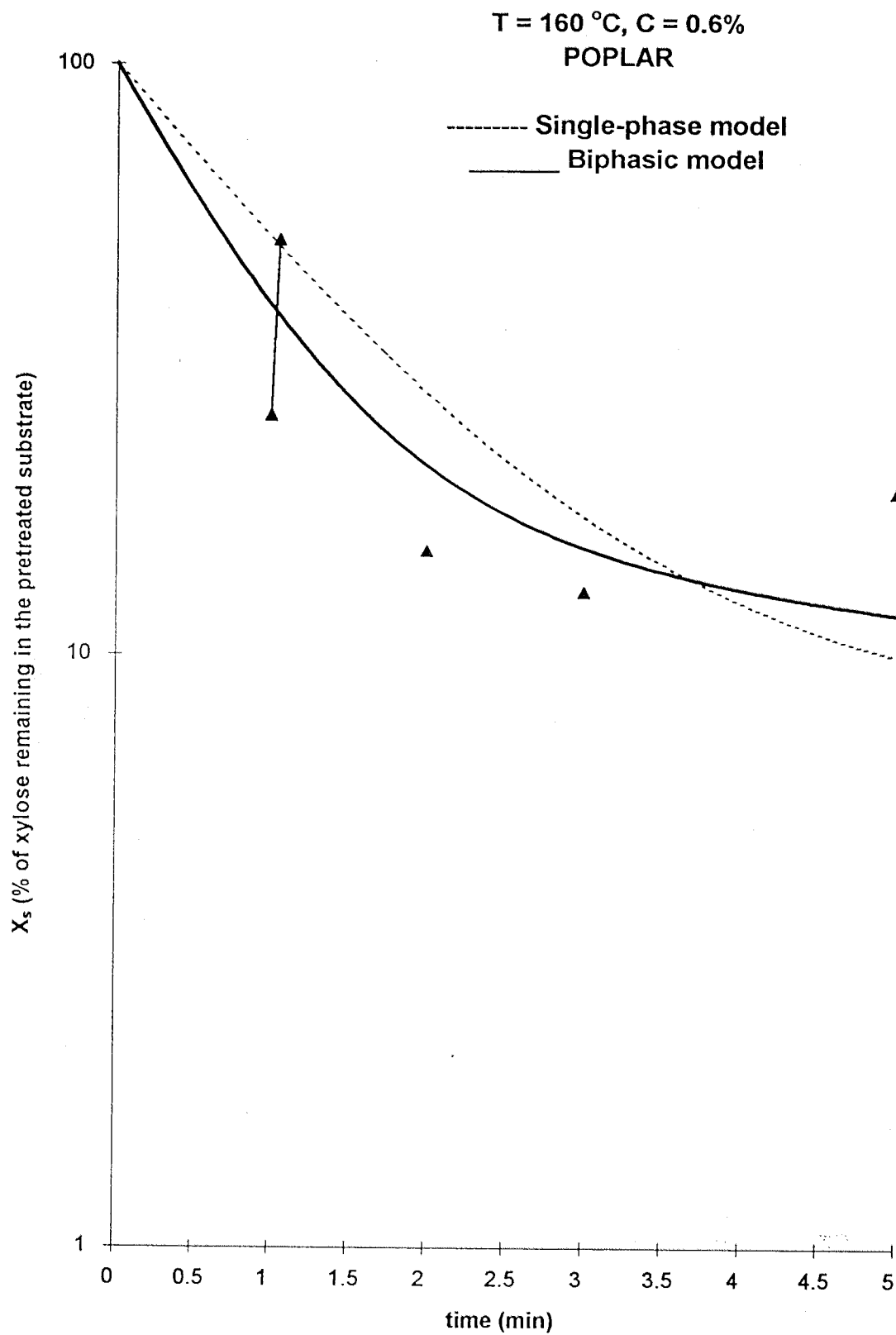


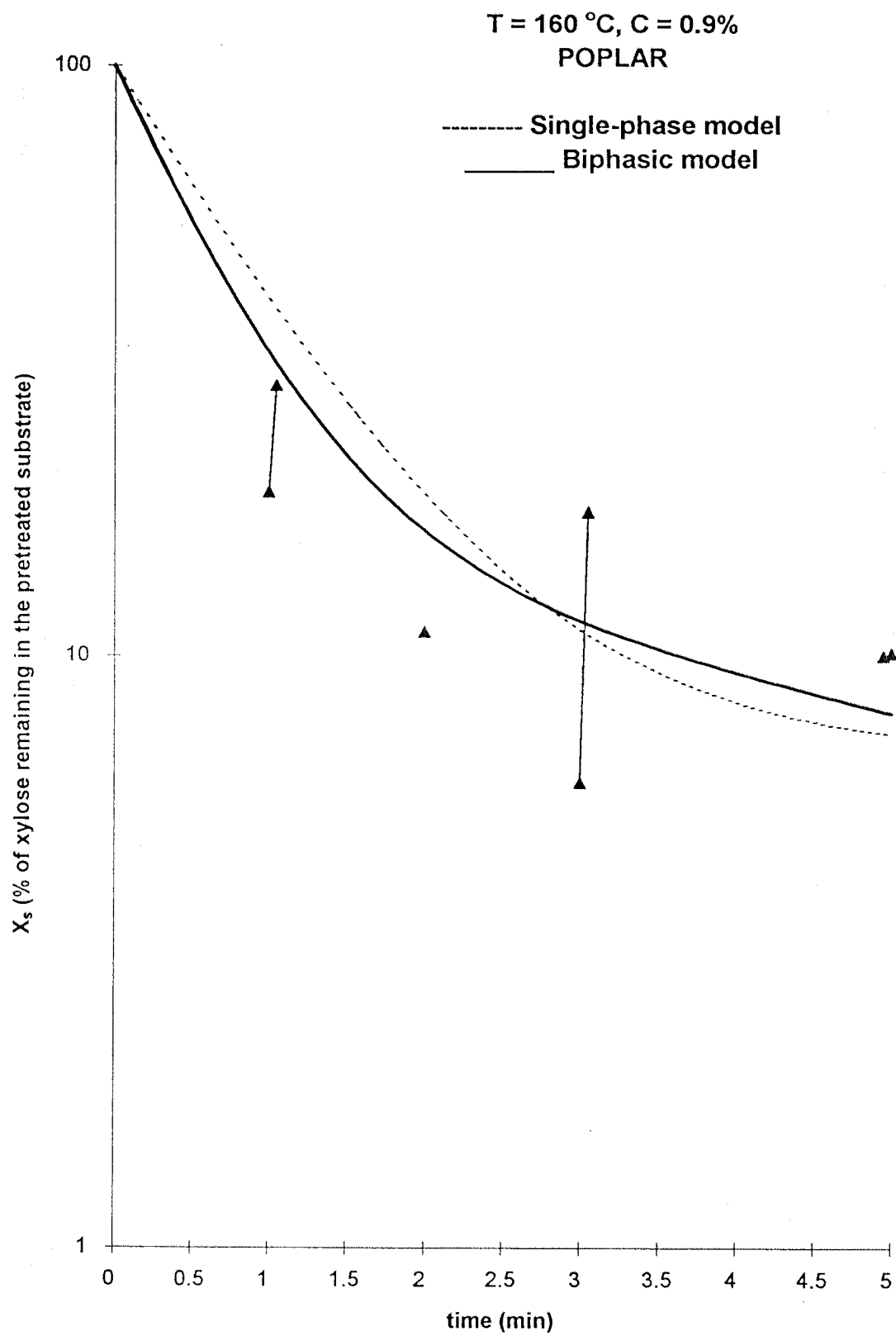
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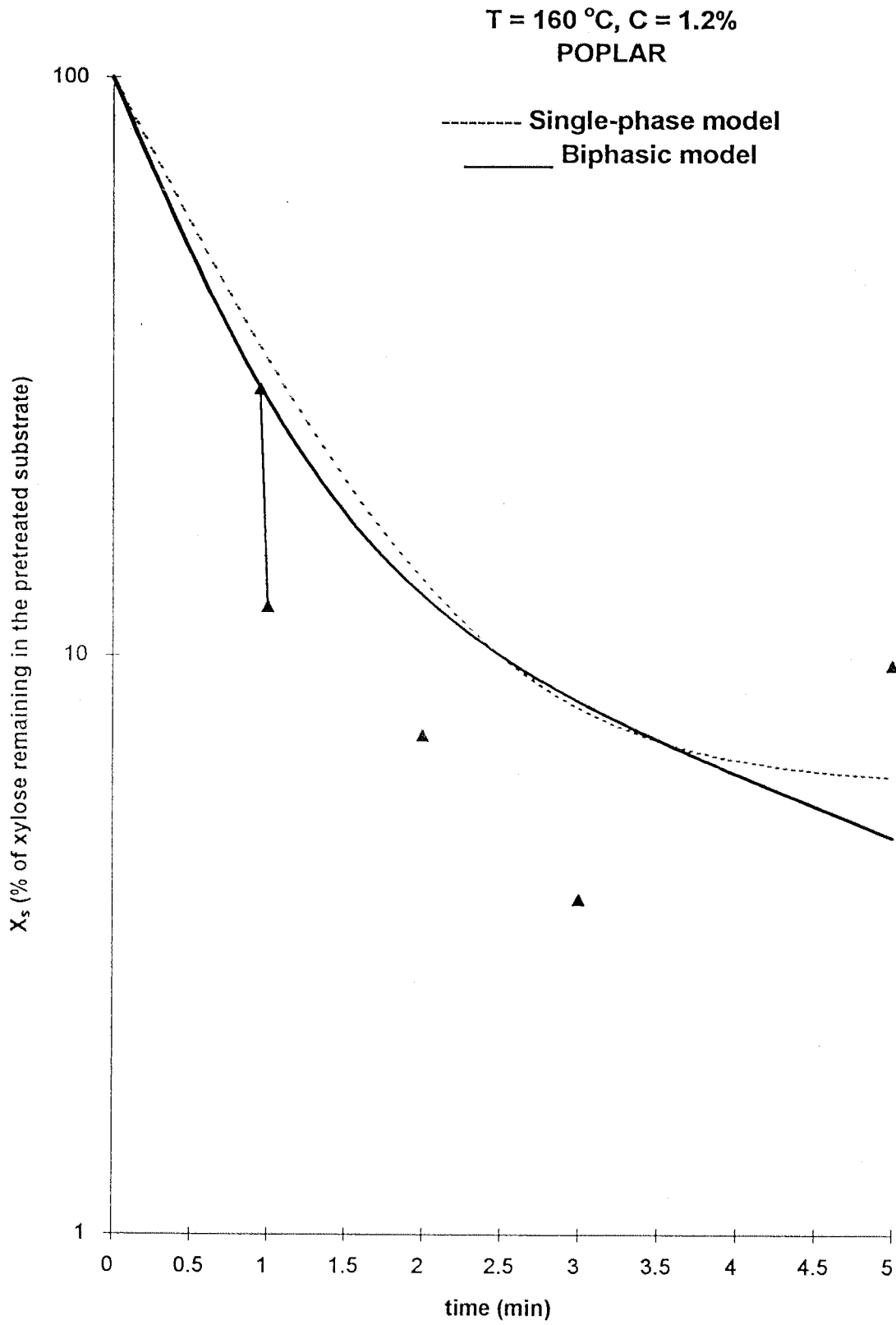
POPLAR

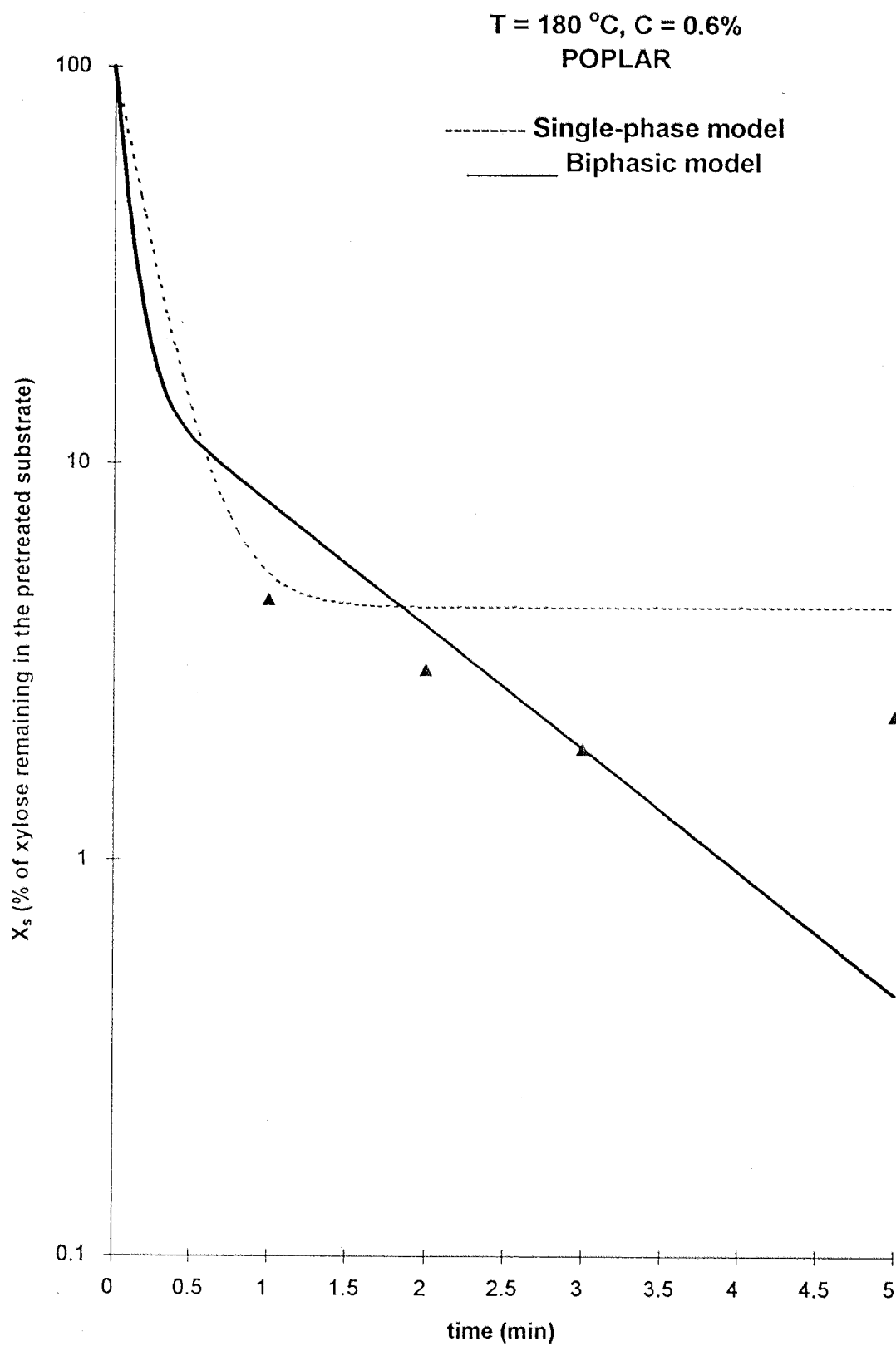






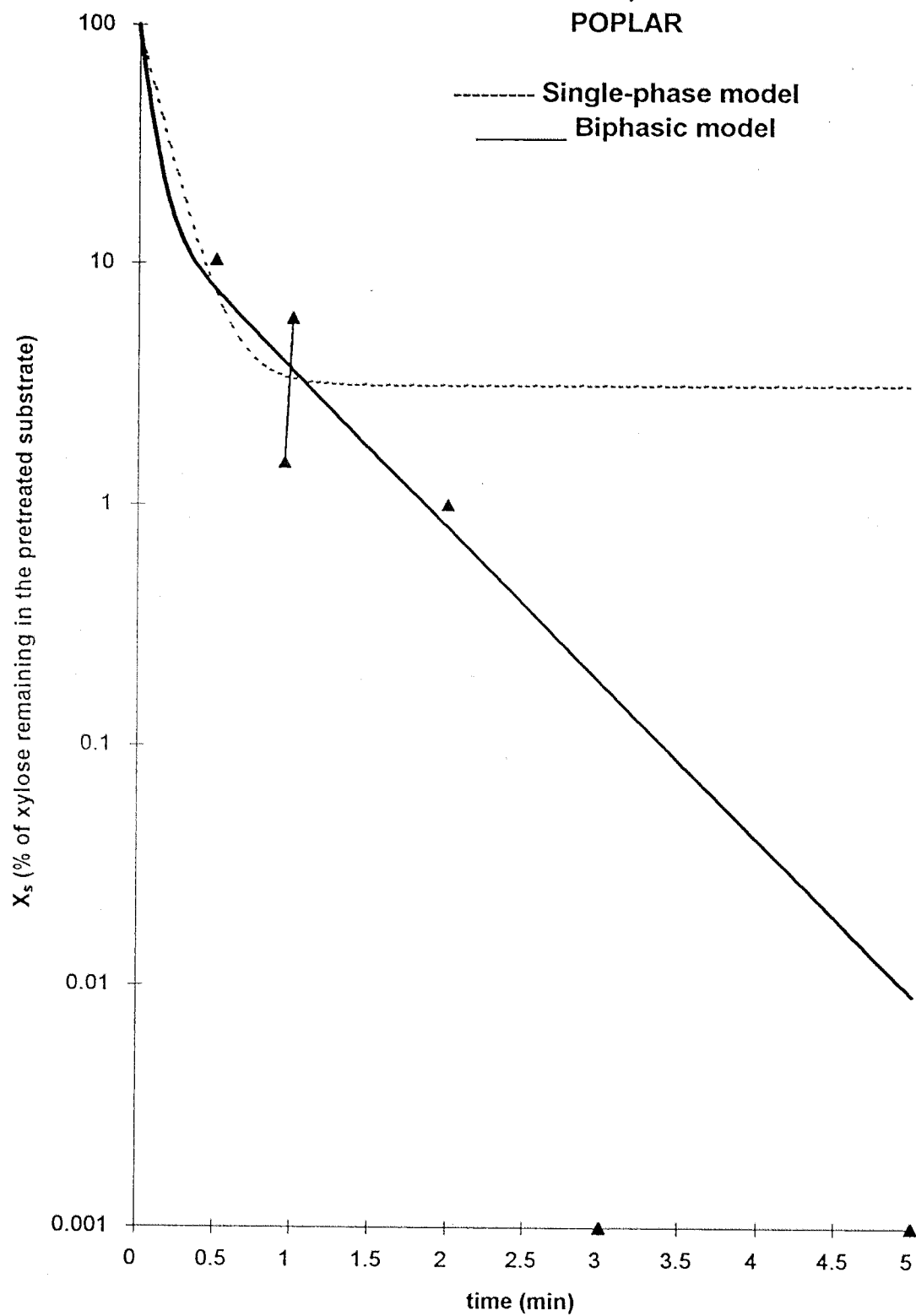






$T = 180\text{ }^{\circ}\text{C}$, $C = 0.9\%$

POPLAR



$T = 180\text{ }^{\circ}\text{C}$, $C = 1.2\%$
POPLAR

